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Bionomics of a Greenbug Parasite Lysiphlebus testaceipes (Cresson)

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BIONOMICS OF A GREENBUG PARASITE, *LYSIPHLEBUS TESTACEIPES* (CRESSON)

BY

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BIONOMICS OF A GREENBUG PARASITE,

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This thesis is approved as a creditable and independent investigation by a candidate for the degree, Master of Science, and is acceptable as meeting the thesis requirements for this degree. Acceptance of this thesis does not imply that the conclusions reached by the candidate are necessarily the conclusions of the major department.

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I INTRODUCTION

The greenbug, Schizaphis graminum (Rondani), is a major pest to grain crops in the Great Plains states. In 1968, 7.3×10^6 acres of sorghum were infested in nine of these states. The loss of sorghum in Kansas alone was estimated at 13.5×10^6 bushels (Harvey and Hackerott 1969b). In 1969 and 1970 the combined South Dakota recorded sorghum yield and control losses were over $\$2.6 \times 10^6$ (USDA 1970 and 1971). The greenbug is an economic vector for diseases of winter wheat, barley, oats, sorghum, and cane. Severe infestations on winter wheat have led to reduced crop cover and soil erosion.

S. graminum has evolved three biotypes, A, B, and C (Wood 1961, Wood et al. 1969). Biotype C has been one of the main limiting factors to grain production, thriving over a wide temperature and plant host range.

The greenbug has migrated northward to the central states each spring (Ainslie 1926, Wadley 1931). There has been a natural time lag which has enabled establishment of the greenbug between its entrance in April and the parasite-predator build-up.

Greenbug populations have been effectively reduced by topical applications of chemicals, often harsh to natural controls and poorly timed. Systemic insecticides are being tested for early, preventative and residual control (Jones et al. 1970, Jones et al. 1971b). Such insecticides will possibly be less damaging to native greenbug predator and parasite populations, allowing their build-up while suppressing the greenbug numbers. A combination of biological and

chemical controls may be the solution to improved production.

The objectives of this project were to contribute to the knowledge of the biology of Lysiphlebus testaceipes (Cresson), considered to be an effective control of the greenbug, and to investigate the influence of certain systemic insecticides in sorghum, on the greenbug populations and on the greenbug predator and parasite populations. The influence of low temperature on successful parasite copulation and emergence was investigated under controlled environmental conditions, and the population studies, under field conditions.

II LITERATURE REVIEW

1. Greenbug Distribution

The greenbug, Schizaphis graminum (Rondani), a major pest to small grains and sorghum, first came into prominence between 1847 and 1852 in Parma and Bologna, Italy (Hunter 1909, Webster and Phillips 1912). After introduction to the Central Americas, the greenbug was collected in the USA in 1882, locality and host not obtainable, and in Indiana from wheat in 1884 (Hunter 1909, Webster 1909). Wadley (1931) described the greenbug distribution as encompassing the Carolinas, the Dakotas, Indiana, Minnesota, Oklahoma, Texas and Wisconsin in the USA as well as regions in Africa, Asia, and Europe. The greatest injury at that time was on oats and on winter and spring varieties of wheat. Dickson and Laird (1969) stated that the greenbug had been a major economic pest of small grains, and occasionally sorghum, from the Atlantic to and including the Great Plains. In 1967, Cramer had ventured to say that the greenbug was a major economic pest to small grain crops throughout the world.

2. Partial Infestation History in the United States

In 1890, the first large outbreak of the greenbug occurred on wheat in Indiana, Tennessee, North Carolina, and Texas (Webster and Phillips 1912).

In 1907, damage to small grains in Oklahoma and Kansas was estimated conservatively at $\$1.5 \times 10^7$ (Ainslie 1926).

In the spring and summer of 1926 damage was noted throughout Minnesota to oats, wheat, and barley (Ainslie 1926). Ruggles and Wadley (1927) estimated that 15 X 10^6 bushels of oats were destroyed.

Spring greenbug infestations in 1942 caused a loss of 61 X 10^6 bushels of grain, including oats, barley, and wheat, valued at greater than \$3.8 X 10^7 in Oklahoma, southern Kansas, and Texas (Fenton 1944).

An estimated loss of 21.9, 2.0 and 0.8 X 10^6 bushels of wheat, oats, and barley respectively, occurred in Oklahoma in the spring of 1950 (Fenton and Dahms 1951).

In the fall of 1958, in Oklahoma, widely used greenbug resistant wheat hybrids were severely injured by the pest. Wood (1961) designated this greenbug biotype B on the basis of host resistance. Comparative studies showed this insect, like its contemporary, biotype A, did little damage during hot summer months (Wood 1961, Dickson and Laird 1969, Wood et al. 1969).

Sorghum, in Africa in 1962 and in Europe in 1964, was attacked by greenbugs in damaging numbers (Harvey and Hackerott, 1969b). Some economic greenbug damage had been noted to sorghum in the USA in 1916 (Kelly 1917, Hayes 1922). In Texas and other Great Plains states, Daniels (1969) and Ward et al. (1970) had reported greenbug populations on the undersides of maturing grain sorghum leaves in August of 1966 and in August and September of 1967.

Severe damage to all stages of sorghum was widespread in 1968, occurring in Arizona, California, Colorado, Kansas, Nebraska,

New Mexico, Oklahoma, South Dakota and Texas (Daniels and Toler 1969, Dickson and Laird 1969, Harvey and Hackerott 1969b, Jackson et al. 1970, Ward et al. 1970, Hackerott and Harvey 1971, Wood 1971a, Hight et al. 1972). An estimated 7.3×10^6 acres of sorghum in nine of the Great Plains states were infested, with the total loss in excess of $\$2.0 \times 10^7$ (Ward et al. 1970).

The greenbug continued to be a pest to sorghum from 1969 through 1972. In South Dakota, 1971, nearly 80% of the grain sorghum acreage or 3.04×10^5 acres had to be treated for greenbug (USDA 1972).

Summer survival of greenbugs had been previously limited to volunteer grains and wild grasses, such as western wheat grass, in moist, often shaded, low-lying acres (Dahms et al. 1954, Daniels 1961a). Although sorghum had been established as a greenbug host, summer field temperatures often exceeding 100°F (37.8°C), had not been conducive to biotype B's survival or reproduction. The upper temperature limit for this biotype's reproduction was reported near 37°C (98.6°F) (Daniels, 1967). Wood et al. (1969) observed it to leave small grains at 80 to 85°F (26.7 to 29.4°C).

The greenbug, observed to reproduce at temperatures exceeding 100°F (37.8°C) and develop at 110°F (43°C) on sorghum in the summer months of 1968, was designated biotype C (Harvey and Hackerott 1969a, Wood et al. 1969, Wood 1971a).

In the falls of 1963, 1964, 1965, 1967, and 1968, newly-emerged winter wheat in the northern Great Plains states was colonized or infested with greenbug (Kieckhefer and Gustin 1967, Kvenberg 1971).

Contrary to conclusions by Fenton and Dahms (1951) and Rogers et al. (1972), the 1968 fall infestations in South Dakota, composed of biotype C greenbugs, had followed a summer of higher than normal temperatures (Kvenberg 1971).

Research indicated that the greenbug could successfully transfer from sorghum to winter wheat (Daniels 1969, Harvey and Hackerott 1969a). It was evident that the greenbug could survive and develop throughout the year in areas of the Great Plains states due to the overlapping of successive crops (Harvey and Hackerott 1969a).

Comparative studies of the three greenbug biotypes, both morphological and physiological, were undertaken (Dickson and Laird 1969, Harvey and Hackerott 1969a, Wood et al. 1969, Kvenberg 1971, Wood 1971a).

3. Greenbug Hosts

At least 78 species of Gramineae, including barley, cane, corn, oats, rye, sorghum and wheat, as well as several other species have been observed as hosts of S. graminum in the USA (Webster 1909, Wadley 1931, Patch 1938, Dahms et al. 1954, Daniels 1960b, Harvey and Hackerott 1969a, Wood 1971a).

4. Greenbug Damage

There have been several aspects to the damage caused by S. graminum. Biotypes A and C are phloem feeders and biotype B is a parenchyma feeder (Wood et al. 1969, Saxena and Chada 1971a). The

injection of salivary enzymes by all biotypes, not the uptake of food, causes geometrically increasing necrosis at the feeding sites (Wadley 1929, 1931; Chatters and Schlehuber 1951; Saxena and Chada 1971b). Damaged cells have been characterized by a severe decline in chlorophyll content and in the rate of photosynthesis (Gerloff and Ortman 1971). This has often resulted in slow maturation reduced root systems; reduced seed size, quantity, and quality; susceptibility of fall planted crops to freeze damage; and the ultimate death of the host (Kantack and Dahms 1957, Ortman and Painter 1960, Hackerott and Harvey 1971). The overall effect has been reduced yield and crop cover, leading to financial loss. In the northern Great Plains reduced crop cover in the winter months has contributed to wind soil erosion.

In addition to direct physical injury the greenbug has been incriminated as a vector of barley yellow dwarf virus, of sugarcane mosaic virus, of western wheat mosaic virus, of oat yellow dwarf virus, and of wheat streak mosaic virus (Ingram and Summers 1938, Oswald and Houston 1953, Connin and Staples 1957, Arny and Shands 1959, Medler and Smith 1960, Orlob and Medler 1961, Shaunak and Pitre 1971). A phoretic relationship was noted with the wheat curl mite (Aceria tulipae Koch), also a vector of the wheat streak mosaic virus (Gibson and Painter 1957).

5. Greenbug Reproduction

The greenbug has been observed to reproduce both asexually and sexually. In the southern states this aphid reportedly reproduces by parthenogenesis year round. Egg production by fertilized females has been primarily limited to latitudes north of the 35th parallel where both sexual and asexual reproduction occurs (Webster and Phillips 1912, Kelly 1917, Wadley 1931). There is some evidence that the greenbug overwinters in the egg stage as far north as southwestern Kansas; farther north the winters are too harsh for the aphid to survive (Kvenberg 1971). In South Dakota, in past summers, the greenbug has been observed to reproduce parthenogenetically.

The greenbug passes through five instar stages. Although parturition does not occur until the last instar, maturation of progeny two generations ahead may occur within the fourth instar nymphs (Wadley 1931).

There are conflicting observations on reproduction rates. Daniels (1963) noted that reproduction was significantly higher at 72 to 75°F (22.2 to 23.9°C) than at 65°F (18.3°C) and that at 50°F (10.0°C) reproduction ceased. Kvenberg (1971) concluded that reproduction decreased as temperature was lowered from 70°F (21.1°C). At 50°F (10.0°C) both B and C biotypes produced an average of 1.0 offspring per day, but the daily offspring number decreased to trace numbers at 45 and 40°F (7.22 and 4.44°C), with all reproduction ceasing at 35°F (1.67°C) and with mortality beginning at 45°F (7.22°C). Kvenberg's (1971) results concur with observations made in

earlier work (Glenn 1909, Hunter 1909, and Webster and Phillips 1912).

At 72 to 75°F (22.2 to 23.9°C), Daniels (1963) observed an average life span of 40 days with an average of 71 progeny per aphid, 3.1 offspring per day. These observations concurred somewhat with Hunter's (1909) at summer temperatures. Hunter observed an average of 55.4 progeny per aphid, 2.43 progeny per day, during an average reproductive period of 22.7 days and an average life span of 35.2 days.

Hunter (1909), dealing with biotype A at summer temperatures observed a prereproductive development time of 7.1 days. Kvenberg (1971) in a comparative study of biotypes B and C observed a development time of 229.3 hr for biotype B and 207.6 hr for biotype C at 63°F (17.2°C). This same author reported a development period of 415.2 hr for biotype B and 420.0 hr for biotype C at 50°F (10.0°C).

A maximum average of 77.8 progeny per aphid, 3.1 per day, were produced by biotype C, while biotype B produced 62.7 offspring, 2.5 per day, at 63°F (17.2°C) (Kvenberg 1971). The reproductive periods of both biotype B and C at this temperature averaged 23.3 days.

At 50°F (10.0°C) biotype B produces a maximum of 13.1 offspring per adult, whereas biotype C produces a maximum of 9.6 offspring with average reproductive periods of 11.1 days and 8.6 days, respectively (Kvenberg 1971).

The appearance of alate forms has been attributed to the quality and quantity of host material, overcrowding, fluctuating

temperatures, photoperiod, drought, and windy periods (Wadley 1931, Kvenberg 1971). Kvenberg (1971) observed that at 63°F (17.2°C) biotype C alates produced 24.11% winged offspring and apterous parents produced 25.8%; and observed that biotype B alates produced 1.04% winged offspring and apterous parents, 1.46% winged progeny.

Wingless viviparous females were more prolific than winged viviparous females (Webster 1909, Webster and Phillips 1912, Kvenberg 1971).

6. Greenbug Migration

Winged greenbugs, transported by southerly winds in spring, have been observed to migrate northward as far as Canada from infested areas in the south (Wadley 1931, Medler and Smith 1960). The migrant' northward advance can occur in successive stages, by convectional currents coupled with intermittent surface winds, or in a single migration, by low level jet streams (Webster 1909, Webster and Phillips 1912, Wadley 1931, Rogers et al. 1972).

Several reasons for migrations have been stated. The migrant winged forms, more restless than the wingless forms, are stimulated to flight by wind and high temperatures, regardless of the host food condition (Wadley 1931). During the 1926 Minnesota outbreak, the alate greenbugs reacted only to southerly winds, causing a continual northward migration (Ainslie 1926). In general, the wind governs both direction and distance of migration and normal dispersion of the greenbug, while the advance of spring northward regulates the

northern limit of greenbug survival (Webster 1909, Webster and Phillips 1912, Wadley 1931).

There are conflicting reports on alate survival without food. Wadley (1931) stated the period was 4 to 6 days in duration at 50 to 60°F (10 to 15.6°C); where as Rogers et al. (1972) stated it was 2 days in duration at spring temperatures. This factor limits the distance the spring migrant can travel in one migration and successfully establish a colony.

7. Greenbug Control

There are four general control methods for S. graminum: natural, plant resistance, mechanical, and chemical.

Reported natural controls primarily included predacious insects in the families Coccinellidae, Cecidomyiidae, Chrysopidae, and Syrphidae; and parasitic insects in the families Braconidae, Eulophidae, Encyrtidae, Pteromalidae, and Cynipidae. Less-noted natural controls were araneids in the family Salticidae, several bird species and empusal diseases. Heavy rains, high temperatures, and advanced grain growth in the spring were also reported as controlling factors (Hunter 1909, Webster 1909, Webster and Phillips 1912, Ainslie 1926, Spencer 1926, Garman 1926, Wadley 1931, Fenton and Fisher 1940, Knowlton 1941, Medler and Smith 1960, Jackson et al. 1970, Muniappan and Chada 1970, Ward et al. 1970).

Plant breeders had developed resistance to biotype A in wheat, barley, oats, and rye grass. CI 9058 wheat and Dickinson Selection

28-A wheat and hybrids containing this germ plasm; Ward, Omugi, and Will barley; and a variety of rye grass are resistant (Boerger 1952, Wood 1961, Wood et al. 1969). Wood et al. (1969) concluded that biotype A no longer occurred in southwestern grain fields. This biotype was not sighted in South Dakota in the 1971 and 1972 growing seasons. Biotype B reproduces normally on Ward barley, on CI 9058 wheat, and on Sel 28-A and its hybrids; but Dicktoo, Will and Omugi barley, Piper sudangrass, Caribou Selection rye, and CI 9058/7*Bison wheat are resistant (Harvey and Hackerott 1969b, Wood et al. 1969, Wood 1971a). All biotype B resistant wheats and wheat hybrids were susceptible to biotype C (Harvey and Hackerott 1969b, Wood et al. 1969). Dickson and Laird (1969) found several barley varieties susceptible to biotype C but according to Wood et al. (1969) Will barley maintained resistance. Dicktoo barley showed some resistance towards both B and C biotypes (Harvey and Hackerott 1969b). Insave F.A. rye proved resistant to biotypes B and C and the Moregrain variety of oats demonstrated some antibiosis to the C biotype (Dickson and Laird 1969, Harvey and Hackerott 1969b). Harvey and Hackerott (1969a) mentioned slight resistance in seedling wheat to the C biotype but this resistance was not sufficient to protect the crop.

It was recently established that seven sorghum varieties and hybrids exhibited varying degrees of tolerance and antibiosis to all three greenbug biotypes (Hackerott and Harvey 1971, Wood 1971a). Most were not comparable to commercial grain sorghums but Sorghum

virgatum (Hack) Staph, SA 7536-1, and Sorghum bicolor (Linnaeus) Moench, P.I. 264453, showed promise of being cross-bred to form acceptable commercial varieties (Wood 1971a).

Prior to 1940 large greenbug infestations were considered uncontrollable (Whitehead and Fenton 1940). Common practices were to burn, or drag chains through infested fields of small grains, apply sprays of kerosene and solutions of whale oil soap, graze closely, destroy volunteer host plants and debris, rotate crops, irrigate prior to planting for increased plant vigor, and use infested crops as hay or pasture (Garman 1926, Hayward 1940, Walton 1943, Griot 1944, Boerger 1952, Daniels 1961a, Wood 1971b).

Topical applications of insecticides have proved more effective for greenbug control but have been detrimental to the natural enemies of the aphid, often toxic to the operator and wildlife, subject to environmental factors such as temperature and wind, often not appropriate for residual action of the insecticide on the foliage, and more than one treatment may be required (Owen et al. 1952, Daniels 1960a, DePew 1964, Ward et al. 1970, DePew 1971a). In more recent years systemic insecticides have been investigated as seed treatments and granular and liquid formulations in broadcast, furrow, band, and side-dress at planting time for early preventative control of the greenbug on small grains and sorghum. In general, granular formulations are more effective, less phytotoxic and less dangerous than liquid formulations and seed treatments (DePew 1964, DePew 1971b).

Granular formulations of disulfoton, at 0.2 to 10 lb active ingredient (AI)/acre rates at planting, have demonstrated a long residual action, significantly reducing field greenbug populations and consequently, often increasing yields of oats, wheat, barley, and sorghum (Hopkins et al. 1957, Daniels 1960a, Daniels 1961b, Jones et al. 1970, Jones et al. 1971b, Wood 1971b). Phorate applied as grnaules at planting time of sorghum and winter wheat, at rates of 1.5, 1.0, and 0.75 lb AI/acre as band and furrow applications, also exhibited residual action against the greenbug and increased yields, although to a lesser degree with sorghum (Jones et al. 1970, Jones et al. 1971a, 1971b). The long residual action of both disulfoton and phorate granules at high rates in furrow and side-dressing treatments was further demonstrated by Hopkins et al. (1957); the insecticides applied at planting not only afforded control to cotton throughout its growing season against the cotton aphid but also to the following fall planted oats against the greenbug. Phorate, applied as seed treatment to winter wheat and spring barley at 1.0 and 0.5 lb/100 lb seed, afforded excellent control for a period of 6 weeks; and disulfoton, as a seed treatment for barley at the same rates, demonstrated similar control (DePew 1964, Wood 1971b). Carbofuran also demonstrated a long residual effect at rates of 1.0 to 0.5 lb AI/acre in a 4- to 7-inch band, resulting in significant decreases in greenbug populations and higher sorghum yields (Jones et al. 1970, DePew 1971a, Jones et al. 1971b). Dimethoate applied in a 4- to 7-inch band at planting gave

a 23 to 40% reduction of greenbugs under the check at the 1.0 and 2.0 lb AI/acre rates (Jones et al. 1971b). Aldicarb under laboratory conditions at rates of .25, .125, and .063 lb AI/acre gave 100% control in a simulated broadcast treatment (Wood 1971b).

Low concentrations of terramycin and tetracyclin, antibiotics, caused reduction in greenbug reproduction and weight (Maniappan and Starks 1971).

8. Lysiphlebus testaceipes (Cresson)

Distribution

The known distribution of Lysiphlebus testaceipes (Cresson) includes the United States, southern Canada, Mexico, Central and South America, Bermuda, Puerto Rico, and East and South Africa (Webster 1909, Sekhar 1957, Schlinger and Hall 1960, Krombein et al. 1967).

The species was first described by Cresson in 1880 and to date has no less than 18 synonyms (Muesebeck et al. 1951).

9. Parasitism of the Greenbug

L. testaceipes, a braconid parasite for which more biological data has been given than for all other North American species combined, has been widely acclaimed as one of the most important checks to S. graminum (Hunter 1909, Webster 1909, Webster and Phillips 1912, Kelly 1917, Wadley 1931, Wilson 1948, Sekhar 1957, Schlinger and Hall 1960, Hight et al. 1972). Two references

indicated it was of minor value in controlling the greenbug (Ainslie 1926, Fenton and Fisher 1940).

The five greenbug instars were all susceptible to the parasite although there was an ovipositional preference for the second and third instars (Webster 1909, Webster and Phillips 1912, Hight et al. 1972).

Aphids parasitized in the first three stadia died without producing offspring at 21 to 32°C (70 to 90°F) (Hunter 1909, Webster and Phillips 1912, Hight et al. 1972). Those parasitized in the third stadium matured and alate forms had deformed wings (Hunter 1909, Webster and Phillips 1912, Hight et al. 1972). The survival period of the aphids parasitized in the third stadium increased at lower temperatures, with possible progeny production (Webster and Phillips 1912, Hight et al. 1972). At 21 to 32°C (70 to 90°F), aphids parasitized in the fourth or fifth stadia produced normal offspring numbers, 3 to 5 progeny per day, until the second or third day after parasitization at which time reproduction declined to zero (Hunter 1909, Spencer 1926, Hight et al. 1972). Webster and Phillips (1912) observed a maximum number of 11 progeny produced in this period. Reproduction ceased 2 days before the greenbug's death (Hunter 1909). Hight et al. (1972) noted that parasitized alate forms were less prolific than parasitized apterous forms. Smaller parasites emerged from aphids parasitized around the third moult; yet these were as competitive and prolific as normal-sized parasites (Webster and Phillips 1912,

Hight et al. 1972). There were conflicting observations on successful emergence from aphids parasitized before the second molt (Webster and Phillips 1912, Hight et al. 1972).

The female parasites carried from 4 to 450 eggs (Webster and Phillips 1912). Parasitization of 95 to 301 aphids, with an average of 38 to 94.6 for individual female parasites, had been observed (Hunter 1909, Sekhar 1957).

Oviposition by L. testaceipes elicited a number of reactions by the greenbug according to Webster and Phillips (1912) and Hight et al. (1972). Often repeated ovipositional thrusts knocked early greenbug instars off the plant, while the fourth or fifth instars frequently fell or walked from the plant when approached or agitated by the parasite. Falling to the hot soil under field conditions in June, July, and August resulted in high greenbug mortality. Frequently a number of aphids fell from the plant in the vicinity of a single aphid agitated by the parasite. Alates reacted quicker than the apterous forms and readily flew when approached a second time by a parasite. Aphids of all stages often raised their abdomens and posterior one or two pairs of legs and moved them vertically, rapidly, several times in succession in reaction to the parasite. Aphids stung three or more times and knocked off the plant usually died.

Spring greenbug populations often flourished to injurious levels without hinderance in the presence of parasites and predators (Hunter 1909, Webster and Phillips 1912, Wadley 1931, Sekhar 1960).

Such infestations were due to an abnormal season, an early migration, or migrations to regions unusually far north where the season was less advanced (Wadley 1931). Each situation was characterized by low temperatures and often by a protracted rain, causing considerable delay in the emergence of the adult parasite; and keeping emerged adults secluded, unwilling to disperse, and unable to oviposit effectively, with little delay in the development and reproduction of the greenbug (Glenn 1909, Webster and Phillips 1912, Sekhar 1960). Increased light intensity and higher temperatures in the spring promoted activity among parasites (Sekhar 1960). Hunter (1909) and Kelly (1917) both attempted distribution of the parasite but the low temperatures at which the greenbug infestations occurred proved detrimental to their introductions.

10. Parasite Dispersal

The transportation of the larval primary parasite within the migrant greenbug is recorded as the parasite's most important means of passive dispersal; of secondary importance was the transportation of adult parasites by winds (Webster and Phillips 1912, Kelly 1917). Walking and flight, stimulated by high temperatures, winds, and sunny weather, allowed dispersal over shorter distances; the parasite appeared content to remain in one place as long as greenbugs were present (Hunter 1909, Webster and Phillips 1912).

11. Parasite Reproduction

The female parasite has been observed to reproduce both sexually and asexually. Progeny by parthenogenesis were almost entirely males but the literature revealed a few females have been produced by this method of reproduction (Hunter 1909, Kelly and Urbahns 1908, Webster and Phillips 1912, Sekhar 1957). Sekhar (1957) and Webster and Phillips (1912) observed only male progeny from virgin parasites.

The progeny sex-ratio of mated females appeared variable. Hunter (1909) observed an average ratio of 65.5♀: 34.5♂; Sekhar (1957) generalized that the number of female progeny was always greater than that of males. The ratio varied with the time of mating following emergence and the age of the aphid host. In early matings, female offspring were preponderant and in later matings the sex ratio of the progeny was approximately equal (Sekhar 1957). Hight et al. (1972) observed that the percentage of females tended to be slightly less for parasites developing from 1 day old aphids. Sekhar (1957) noted copulation of L. testaceipes occurred only after 85 min following emergence and drying. Kelly (1909) observed mating within 1 min of emergence; mated females were observed to successfully oviposit in S. graminum within 5 min and virgins within 4 min of emergence. Webster and Phillips (1912) simply stated that under favorable conditions oviposition commenced within a few hours after emergence. Sekhar (1957) observed only single matings with females and multiple matings, up to 19, with males.

12. Parasite Temperature Studies

Aphidiinae have the ability to under go a diapause condition in winter or summer or both. L. testaceipes may under go a weak diapause condition in northern United States and Canada (Schlinger and Hall 1960). Overwintering of L. testaceipes has often been referred to in terms of hibernation of larvae or pupae within the skin of the dead aphid or mummy (Webster and Phillips 1912). They survived extreme cold in relatively small numbers, emerged and reproduced slowly in the spring with a continuous temperature above 56°F (13.3°C) (Webster 1909, Webster and Phillips 1912, Clausen 1951). According to Hunter (1909) the adult parasites emerged at quite low temperatures when extremities ranged between 60 and 3°F (15.6 and -16.1°C) with the mean about 35°F (1.67°C). L. testaceipes failed to overwinter in Minnesota or did so in restricted environments (Wadley 1931). Hibernation has occurred in central California, Indiana, Oklahoma, and Kansas (Webster and Phillips 1912, Wadley 1931, Schlinger and Hall 1960). Excessive moisture during hibernation was detrimental to the successful emergence of the parasite as it enhanced the growth of mold (Webster and Phillips 1912, Jones and Balsbaugh, personal communication).

There were conflicting observations on the lower temperature limits of oviposition. Webster and Phillips (1912) observed the lower limit to be 56°F (13.3°C) but Sekhar (1960) reported total inactivity at 57°F (13.9°C). Hunter (1909) observed oviposition at 38°F (3.33°C), 40°F (4.44°C), and feeble attempts at 35°F

(1.67°C). Both Webster and Phillips (1912) and Hunter (1909) attested to L. testaceipes' ability to survive for extended periods below 32°F (0°C) and oviposit with the warmer temperatures. Activity increased with higher temperatures and was normal at 70°F (21.1°C), but at temperatures greater than 70°F (21.1°C) the parasites had a tendency to take to flight (Hunter 1909). Hunter (1909) and others noted multiple oviposition within a single aphid, but observed in all cases that a maximum of only one adult emerged.

There were a number of observations on development rates. Webster (1909) recorded a development period of approximately 10 days and Hunter (1909) correlated a range of mean temperatures from 35 to 72.4°F (1.67 to 23°C) with a range of development periods from 140 to 11 days, stating the minimum time to be 7 days. Hight (1972) stated that the egg-mummy period varies with each temperature level and the developmental stage of the host; there was a general tendency for 1 day old aphids to take a day longer to develop into mummies at temperatures between 21 to 32°C (70 to 90°F). Mummies at the 21°C (70°F) level required an average of 2 days longer in development and between 21 to 32°C (70 to 90°F) the egg-mummy period required from 7 to 10 days regardless of temperature, and egg-adult periods varied from 10 to 14 days, but parasites developing at 21°C (70°F) took slightly longer (Hight et al. 1972).

III LOW LEVEL TEMPERATURE STUDIES (LABORATORY)

1. MATERIALS

(a) Insects and Host Material

Schizaphis graminum (Rondani) belongs to the family Aphididae in the order Homoptera. Prior to 1963 most literature classified it in the genus Toxoptera. It is often referred to by the common name, greenbug or spring grain aphis. Biotype C specimens were imported from three sources, Oklahoma, Nebraska, and Kansas between early and mid-December of 1971 at the beginning of the study. At this time, biotype B specimens were also received from Oklahoma. Lysiphlebus testaceipes (Cresson) belongs to the family Braconidae in the order Hymenoptera but prior to 1961, literature refers to it, either as Lysiphlebus or Aphidius testaceipes. Specimens, both mature and immature, were imported from a colony at Oklahoma and were verified by B. D. Burks, Plant Pest Survey and Detection, United States Department of Agriculture, Agricultural Research Service, prior to the study.

Of the winter wheat varieties grown in South Dakota, Wenoka was chosen as the host for rearing the greenbug colony, and as the host of parasitized aphids in the temperature studies. This variety was the most readily available when the greenbugs were received and proved to be an acceptable host.

Daniels and Jackson (1968) found that aphids reared on winter wheat in the laboratory were heavier than those on grain sorghum.

(b) Growth Chambers

There were two environmental control chambers used in this study and both units were housed in laboratory space at South Dakota State University, Entomology-Zoology Department at Brookings, South Dakota.

One chamber (Model PGC-78) was manufactured by Percival Refrigeration and Manufacturing in Des Moines, Iowa.

Temperature control capabilities of this unit ranged from 40 to 110°F (4.44 to 43°C) with a temperature uniformity of ± 3F°. A Paragon® timer for diurnal settings and a Part-low® 24-hour recorder and temperature control were available on the cabinet for temperature control. The former in conjunction with Penn® thermostats were utilized in this study (Figure 1).

The unit was equipped with two banks of eight fluorescent lights each and one bank of 10 incandescent lights. Each bank was controlled by a separate Paragon® timer (Figure 1).

Humidity within the cabinet was produced by a Walton® atomizer unit placed on the floor of the cabinet (Figure 2), and controlled by a human hair sensor humidistat inside the cabinet. The humidifier received distilled water by gravity flow through a length of polyethylene tubing from an externally mounted carboy on top of the cabinet (Figure 2).

There were 14 square feet of total bench space within the cabinet. A maximum of approximately 75% of this area was utilized.

Fig. 1.--Controls for the Percival environmental cor
photoperiod and temperature controls, timers and therm

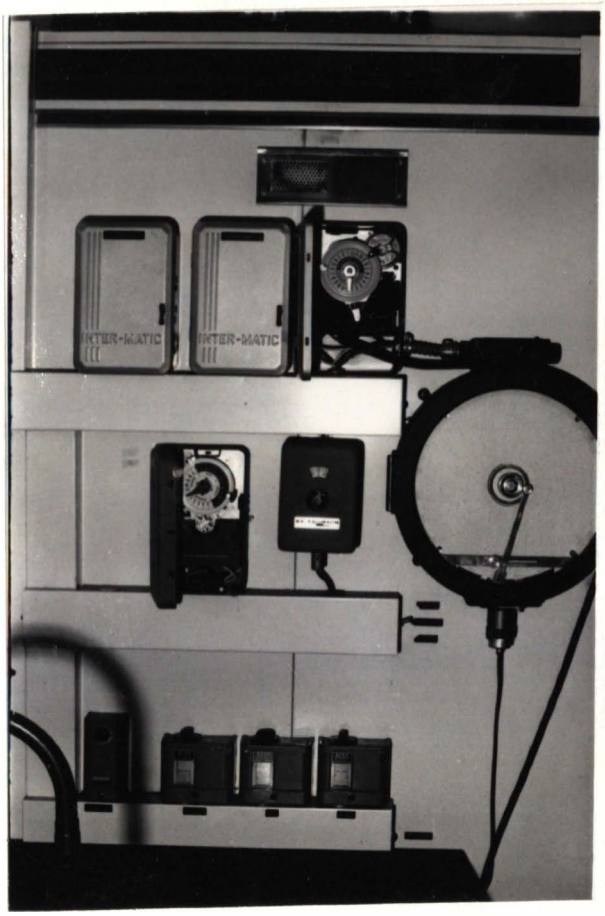
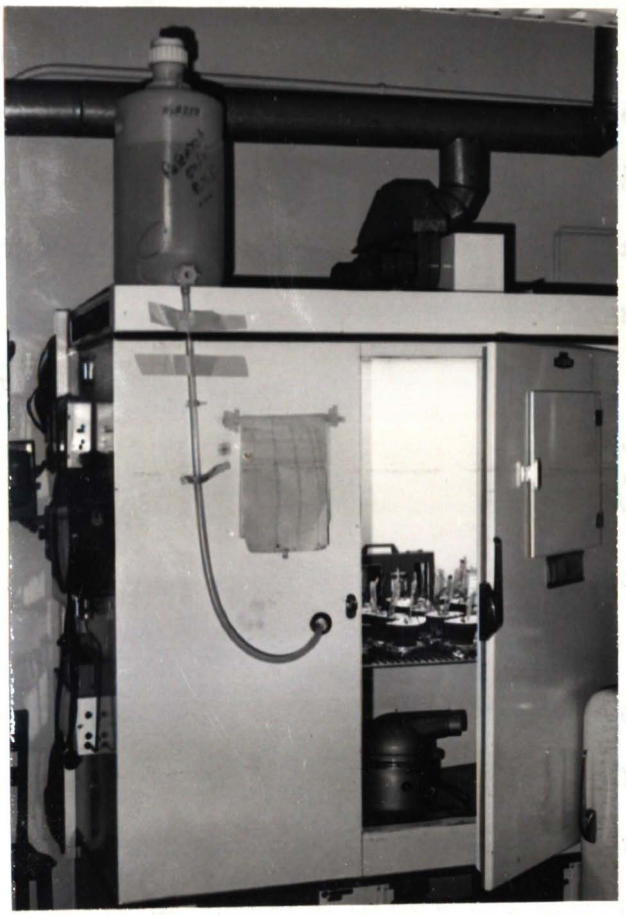

INTER-MATIC
INTER-MATIC

Fig. 2.--Percival environmental control chamber with external mounted water source connected to the Walton® atomizer, on the floor of the chamber, by polyethylene tubing.



The second environmental control chamber (Model Cel 255-6) was manufactured by Sherer-Gillett Company in Marshall, Michigan.

The temperature control capabilities ranged from 40 to 110°F (4.44 to 43°C) with a temperature uniformity of ± 3 F°. A Paragon® timer for diurnal settings with White-Rodgers® thermostats were the means of temperature control (Figure 3).

There were two banks of four flourescent lights each and two banks of two incandescent bulbs each. The photoperiod was controlled by a Paragon® timer (Figure 3). A three way manual switch allowed full or half use of the flourescent lighting and an on-off switch controlled the incandescent banks (Figure 4).

Humidity within the cabinet was produced by a Thru-Wall Hermidifier®. This device was controlled by a Penn® humidistat and was fed distilled water by gravity flow through polyethylene tubing from an externally mounted carboy.

There were 12 ft^2 of total bench space within the cabinet, including both the wire-rod steel plant bed and the floor of the chamber. Approximately 92% of this area was utilized.

Temperature and relative humidity within the cabinets were checked twice daily at 12-hr intervals with the aid of a thermometer and a recording 7-day Hygro-Thermograph® (Figure 5). The Hygro-Thermograph® needles were set according to three average readings on a Sling-Psychrometer.

A Ben-Hur® freezer unit was used to cool adult parasites for manipulation.

Fig. 3.--Sherer environmental control chamber: photoperiod
temperature controls, clocks and thermostats.

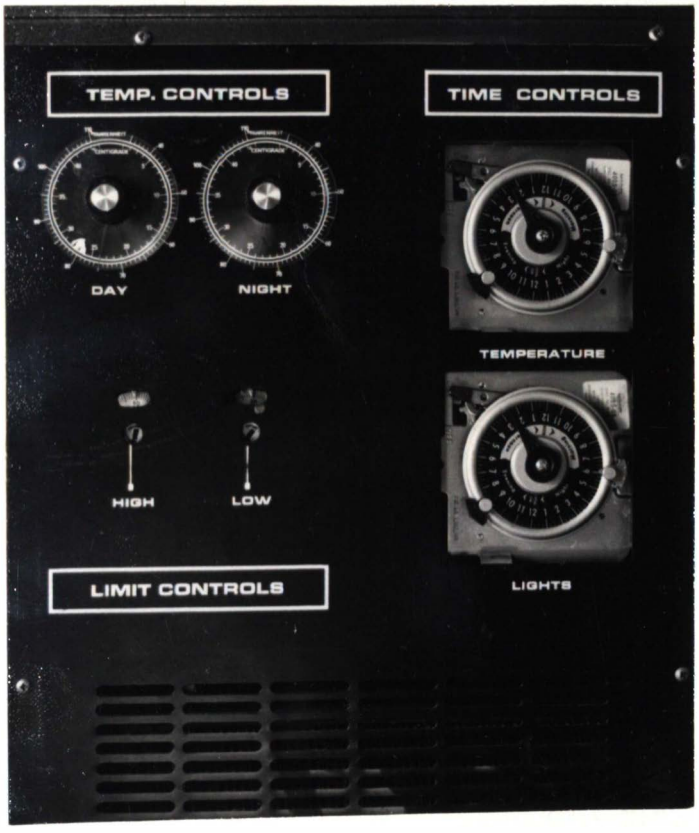

TEMP. CONTROLS
TIME CONTROLS
DAY
NIGHT
TEMPERATURE
HIGH
LOW
LIMIT CONTROLS
LIGHTS

Fig. 4.--Sherer environmental control chamber: fluorescent incandescent light control switches.

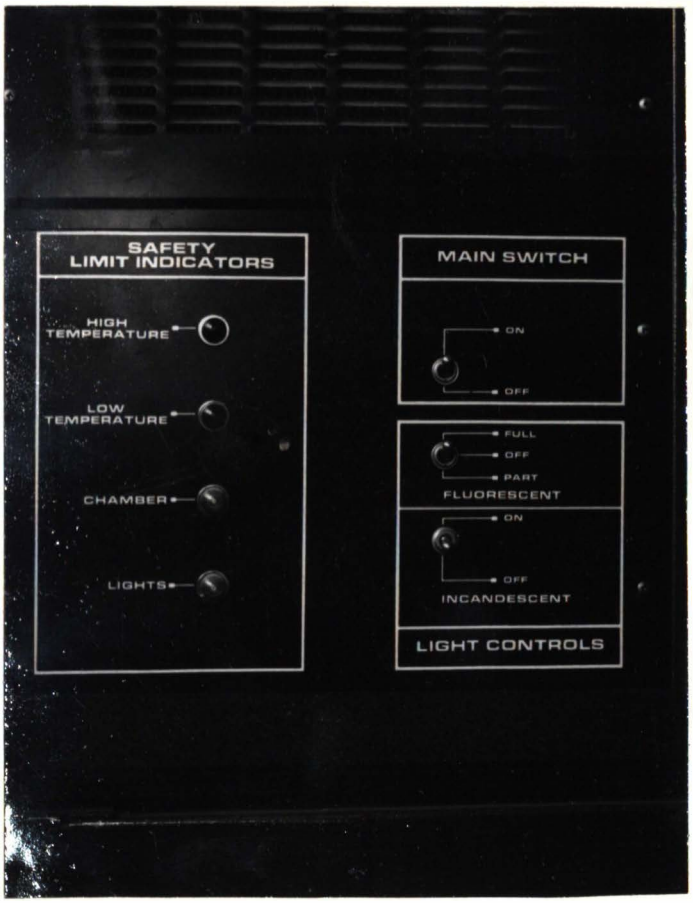

SAFETY
LIMIT INDICATORS
HIGH
TEMPERATURE
LOW
TEMPERATURE
CHAMBER
LIGHTS
MAIN SWITCH
ON
OFF
FULL
OFF
PART
FLUORESCENT
ON
OFF
INCANDESCENT
LIGHT CONTROLS

Fig. 5.--Thermometer and recording 7-day Hygro-Thermograph within growth chamber.


Comfortmeter
120
100
80
60
40
20
0
130
110
90
70
50
30
10

(c) Additional Equipment

The cages used for confining stock colonies of aphids on host plants and preventing insect and mite contamination of winter wheat seedlings were cellulose nitrate cylinders with a diameter of 6.5 cm (2.6 inches) and a height of approximately 30 cm (11.8 inches) (Figure 6). The top of the cylinders and two pairs of 4.0 X 2.5 cm (1.6 X 1.0 inches) portals were covered with bolting cloth. Chada (1962) recommended cellulose nitrate as a cage material and similar cages were used by Kvenberg (1971).

A second cage model, more appropriate for confining aphids in the spring and summer months, was comprised of bolting cloth, a wooden frame, and a plate glass window (Figure 7).

Cages used to confine aphids and parasites to individual leaves were constructed of 5 cm (2.0 inches) lengths of 3/8 inch (1.0 cm), inside diameter, Tygon® tubing. Each end of the cage was sealed by a stopper composed of absorbent cotton and silicone gel crystals wrapped in fine nylon mesh. The Tygon® cages were secured to stakes, wooden tongue depressors, by elastic bands (Figures 8 and 9).

The greenbug colonies were maintained on winter wheat seedlings grown in 4-inch pots while individual plants for aphids in the temperature studies were grown in 3-inch pots. The 3-inch pots were placed in saucers covered with aluminum foil to prevent excessive evaporation into the chamber (Figure 9).

Mummies and newly-emerged adult parasites were confined in

Fig. 6.--Cellulose nitrate cage used to house biotype B stoc
colonies.

Fig. 7.--Cages used to house biotype C stock colonies.

Fig. 8.--Tygon® tubing cage, silicone gel absorbent cot
stoppers, used to house parasitized aphids on a winter whe
section.

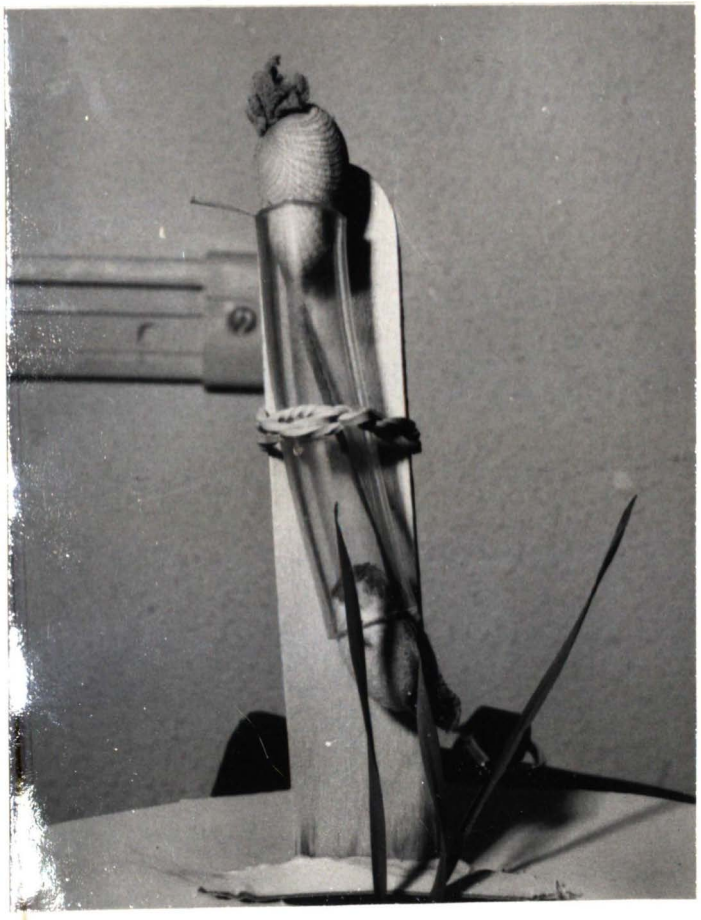
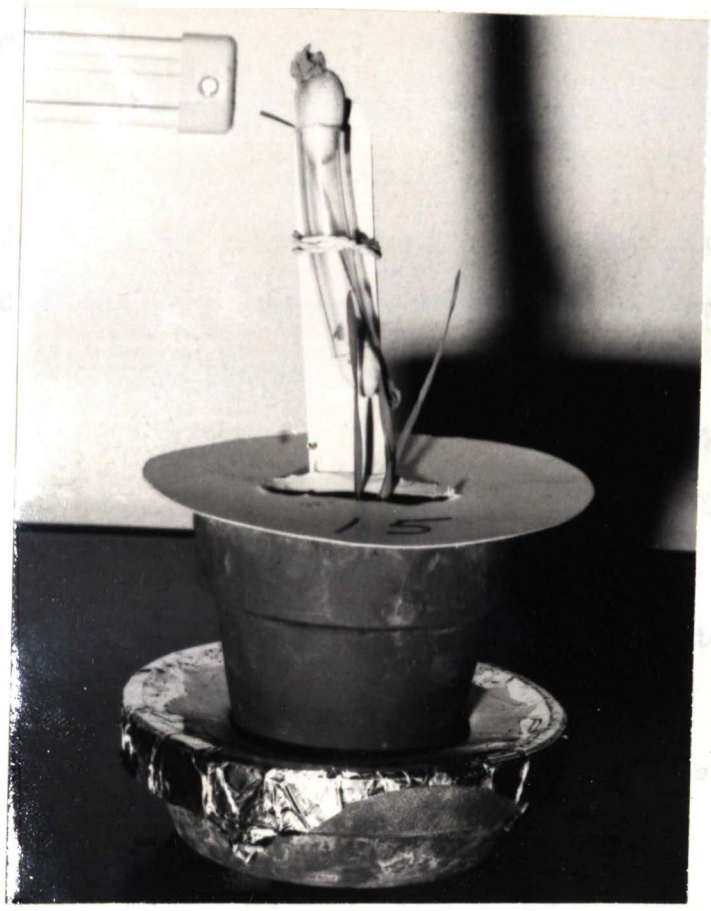


Fig. 9.--Aluminum foil covered saucer and Bristol board cover.


15

No. 00 gelatin capsules produced by Eli Lilly and Company, Indianapolis. Capsules were retained in marked plastic bags (Figure 10).

Liquefied compressed CO_2 gas, contained in a tank with a Matheson® pressure gauge, was used to anaesthetize parasites in early manipulations. The gas was inserted into the Tygon® cages by a hypodermic needle taped to a length of Tygon® tubing attached to the gauge nozzle (Figure 11).

Both aphids and parasites were manipulated with a fine camel's hair brush and leaves with mummies attached were sectioned with scissors or an industrial purpose razor blade.

Lab-Tek® disposable petri dishes lined with moist Garland® Sof-Knit® paper towels were used to germinate all seedlings to insure a degree of uniformity in plant age.

A Weston Illumination Meter®, Model 756 was used to measure light intensities.

A circle, 11.5 cm (4.5 inches) in diameter, of white Bristol board with two central parallel slits was placed on top of the flower pots in the parasite colony and the temperature studies. The stake passed through one slit and the winter wheat seedling through the other (Figure 9). The purpose of the disc was to prevent the loss of aphids and mummies, which might otherwise drop from the leaf into the soil, while the cage or leaf was being replaced.

Fig. 10.--No. 00 gelatin capsules contained in labelled bags.

Fig. 11.--Cylinder of liquefied compressed CO_2 gas and Matheson® pressure gauge, Tygon® tubing and hypodermic ne attached.

2. METHODS

(a) Establishment of Colonies

Greenbugs were established on Wenoka winter wheat at the Northern Grains Insects Research Laboratory, Brookings, South Dakota. Specimens from each state were isolated in separate cages, labelled according to origin.

After the individual colonies became established, color variation was noted among the greenbugs derived from the Oklahoma package. On closer inspection, both biotypes B and C appeared to be present based on information reported by Kvenberg (1971). The biotypes were separated into different cages. Samples of both specimens were sent to K. J. Starks at Oklahoma State University who felt it probable that both biotypes were present.

The colony derived from Kansas specimens was arbitrarily chosen for the experiments with L. testaceipes. The colonies from Oklahoma and Nebraska, except for the biotype B colony, were destroyed. Aphids used in this study were alate biotype C forms (Figure 12).

On receipt of adult and immature forms of L. testaceipes in early December from Oklahoma, the parasites were placed in large cages (Figure 7) containing winter wheat seedlings infested with greenbugs. Greenhouse space was furnished by the Plant Science Department, South Dakota State University, Brookings, South Dakota. This colony failed to become established due to unforeseen difficulties.

A second package of parasites was received in mid-April from

Oklahoma and the specimens were transferred to a laboratory area of the Entomology-Zoology Department, South Dakota State University, Brookings, South Dakota.

Limited numbers of emerged female and male parasites were placed in Tygon® cages with approximately 10 late instar or adult greenbugs. The mummies in the package, on separation from the clump of host material, were individually placed in gelatin capsules. The capsules were then placed on Scotch® tape strips. On emergence, virgin females and males were placed into Tygon® tube cages with approximately 10 greenbugs on Wenoka winter wheat for copulation and oviposition.

(b) Maintenance and Management

I. Greenbugs

When the project was initiated, both the plants and the stock colony of aphids were maintained at the Northern Grains Insects Research Laboratory's greenhouse complex until July 22. The plants and insects were subject to winter and spring photoperiod conditions. The temperature was controlled by a thermostat set at 70°F (21.1°C) but temperatures fluctuated between 52°F (11.1°C) and 109°F (42°C). The relative humidity was not controlled in the greenhouse but condensation in the cellulose nitrate cages indicated that relative humidity within the cages was high.

In late July, the plants and stock colony of greenbugs were transferred to a laboratory of the Entomology-Zoology Department,

Fig. 12.--S. graminum, biotype C adult alate ♀ (25 X).

South Dakota State University, Brookings, South Dakota. Both winter wheat seedlings and greenbug colonies were maintained on bench space by a window. The outside light was supplemented by four 96-inch cool white fluorescent lamps and four 150-watt, 120-volt incandescent lamps suspended approximately 73 cm (28.7 inches) above the 4-inch pots and produced a total of 460 ft-c (Figures 13 and 14). The photoperiod of the supplementary lighting was 10 hr, regulated by a Paragon® timer. The colonies were maintained here through the summer months until early fall and were subject to daily and seasonal fluctuations of temperature. During periods when the temperatures were above 80°F (26.7°C), the incandescent lights were switched off. Temperatures were recorded twice daily at 12-hr intervals and varied between 63°F (17.2°C) and 96°F (35.6°C) at this location; relative humidity again was not a controlled factor. The relative humidity at both locations fluctuated between 31% and 84%, and was highest just after watering the plants.

In the period of higher temperatures, from late July to late August, both colonies appeared to be declining in numbers, and were transferred to the Sherer growth chamber set at 70°F (21.1°C), with a photoperiod of 15 hr and a light intensity of approximately 500 ft-c.

Initially, approximately 17 winter wheat seedlings, of even germination and similar height, were transferred from moist paper towels to 4-inch pots but transpiration and consequent condensation on the inside of the cellulose nitrate cages, indicating a relative

Fig. 13.--Laboratory bench, Entomology-Zoology Department, South Dakota State University; outside light supplemented by fluorescent and incandescent lighting.

Fig. 14.--Greenbug host, Wenoka winter wheat seedlings in
4-inch pots.


20
40
60

humidity approaching 100% inside the cages, caused mold formation on the leaves and on the greenbugs. The problem was alleviated by halving the number of winter wheat seedlings per cage and using the bolting cloth, wooden-framed cages (Figure 7). Greenbugs were transferred to the winter wheat seedlings, approximately 13 days old. Biotype B continued to be maintained in several cellulose nitrate cages on the Wenoka winter wheat seedlings.

The aphids were transferred to new host material when the contaminated plants showed a pronounced yellowing and large numbers of aphids ceased to feed and began to search about the cage. The colonies were maintained in the individual cages on the wheat for a period of 10 days depending on the initial aphid population and the temperature of the rearing area. Transferral took place over a white tray, in an area away from the growth bench and was attained by cutting the dying host material at the soil level. The cage with the host material was then placed over the fresh seedlings. Those aphids which had dropped onto the tray during the transferral were individually placed on the seedlings with a camel's hair brush. If both biotypes needed new host material, to minimize contamination, the tray was washed and dried after the transfer of biotype C and then biotype B was transferred to its new host material. After the cellulose nitrate cage containing biotype B had been put into place, a layer of fine white sand was poured about the base to prevent escape. A few days after transfer, when the greenbugs had moved to the new host material, the dead leaves were removed. Careful

scrutiny for color variation during transfer indicated no contamination was occurring.

To maintain high alate numbers in the colonies, as the alate form was used in the parasite studies for uniformity, the colonies were kept under crowded conditions (Kvenberg 1971).

II. Parasites

Individual leaves with the greenbug hosts were placed diagonally in the Tygon® cages to facilitate the parasite's search for its host (Figure 8). From the stock colony 10 alate aphids were placed directly onto the leaves with a camel's hair brush. Before the parasites were introduced into the cages, all aphids were checked to insure that they were alive. Dead alates, never more than two, were replaced.

Condensation on the insides of the Tygon® cages was a problem and was magnified during the temperature studies. As mentioned previously, silicone gel had been placed in the cotton stoppers to absorb the excess moisture. This did not alleviate the problem, even with frequent changes of the stoppers. Consequently, the cages, as well as the stoppers, were renewed at intervals.

For the maintenance of the parasite stock colony, for the preliminary studies, and for the temperature studies, alate mummies (Figure 15), produced in the same ovipositional period, were obtained from the stock colony of parasites. The leaves with mummies attached were sectioned. Each leaf section with a mummy, or the mummy alone

Fig. 15.--Empty mummy from which L. testaceipes emerged (

where it had become detached from the leaf, was placed individually into a gelatin capsule until emergence. A similar method was used by Jackson et al. (1971) with Aphelinus asychis (Walker). The capsules were attached to Scotch® tape and labelled according to origin of the mummy and were checked daily for emergence under parasite colony conditions.

The colonies were maintained in a growth chamber at 75°F (23.9°C) ± 3F° and 55 to 100% relative humidity. The photoperiod was set at 15 hr using only fluorescent lighting with a light intensity of 1350 ft-c.

Prior to mating, parasites were sexed and retained individually in marked gelatin capsules. Females (Figure 16) were differentiated from the males (Figure 17) on the basis that females have 13 or less antennal segments (Figure 18) and pointed abdomens (Figure 19), whereas males have 14 or more antennal segments (Figure 20) and blunt or rounded abdomens (Figure 21) (Hunter 1909). The abdominal characters were the major mode of differentiation using a binocular dissecting microscope.

During the copulation period, Spencer (1926) noted that in order to sense the female, the male had to be within 7 to 10 mm (.3 to .4) inches of the female. He also noted, as did the author, that the female when approached usually runs away with the male pursuing. It therefore seemed ideal to confine the mating parasites in the capsule, of which the dimensions were 2.4 cm (.9 inches) in length and 8.0 mm (.3 inches) in diam, for the copulation period.

Fig. 16.--L. testaceipes, ♀ (35 X).

Fig. 17.--L. testaceipes, ♂(35 X).

Fig. 18.--L. testaceipes, ♀, showing 13-antennal segmen
(60 X).

Fig. 19.--L. testaceipes, ♀, showing pointed abdomen (8

Fig. 20.--L. testaceipes, ♂, showing 14-antennal segment

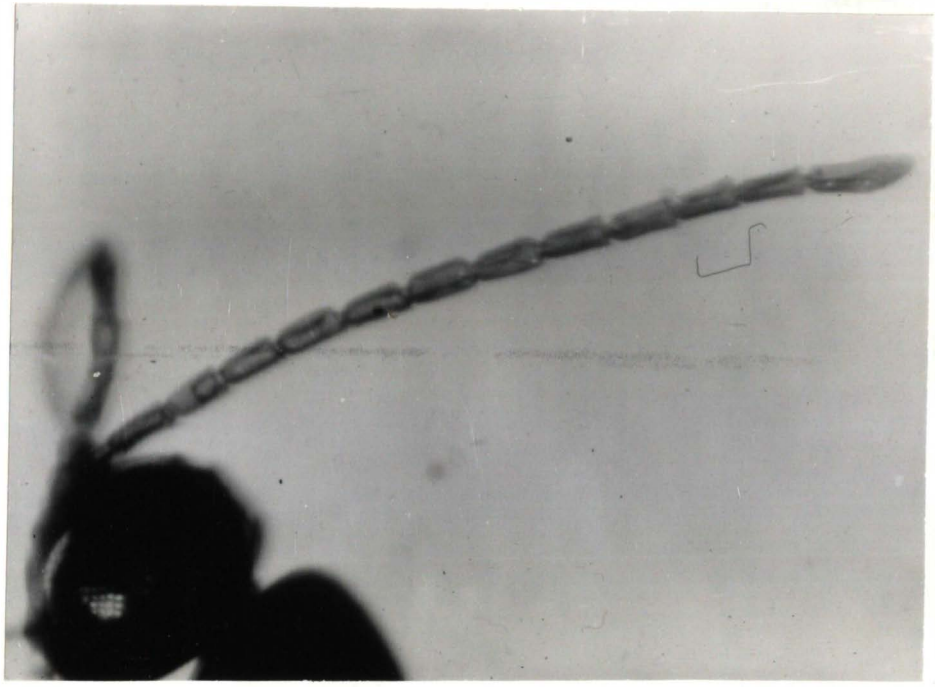


Fig. 21.--L. testaceipes, ♂, showing rounded abdomen (

There were three methods for manipulating newly-emerged adult parasites. In the first method, emerged adults in the Tygon® cages could be handled efficiently by placing the cages with the potted plant into a Ben-Hur® freezer at 10°F (-12.2°C) for 30 sec. On removal from the freezer the parasites were immobile and could be quickly removed from the cage and placed individually into gelatin capsules with a camel's hair brush. If the seedling was left too long in the freezer it died. At the time of mating the capsules containing the parasites involved were placed into the freezer for 30 to 60 sec, after which the immobile insects were placed into a single capsule for copulation. The second method was to anaesthetize the emerged parasites with CO_2 gas put into the cages with a hypodermic needle inserted between the stopper and cage wall at the top of the cage. The parasites were removed and placed individually into gelatin capsules. The cages and stoppers were then removed for a short period to allow full aeration in the area of the remaining mummies. For copulation the parasites were placed into one capsule by careful manipulation of capsule halves containing parasites. The third method, the basic method used in colony maintenance and in the temperature studies of L. testaceipes, was to utilize the phototropic character displayed by the parasite. Holloway (1913) had manipulated Trichogramma minutum (pretiosa) Riley with a camel's hair brush and the phototropic character of the parasites. The top of the Tygon® cage was pointed toward the light source, the upper stopper was removed, and the emerged parasites were removed and placed gently

into gelatin capsules with a camel's hair brush as they reached the top of the cage.

The male and female marked capsules were kept in separate plastic bags, at the temperature level at which copulation and oviposition was to occur, for a minimum period of 2 hr before copulation.

After preliminary studies, it became the practice to mate a single female with three males for a 2 hr copulation period. This copulatory group was maintained during the oviposition period. At the conclusion of the copulatory period, the parasites were maneuvered into one capsule-half using their phototropic character and then allowed to disperse from the capsule into the Tygon® cage containing the aphid hosts for oviposition. The oviposition period was 5 hr. Upon completion of oviposition the upper stopper of the Tygon® cage was removed and the parasites, attracted to the open top of the cage by the lights in the chambers, were lifted away with a camel's hair brush.

The manipulations of the parasites, copulation, and oviposition all occurred in a chamber at 75°F ± 3F° (23.9°C) for maintenance of the colony population, and at 75°F ± 3F° (23.9°C), 65°F ± 3F° (18.3°C), or 55°F ± 3F° (12.8°C) according to the temperature level at which the parasite was being studied. During periods of manipulation in the cabinets, the temperature was held within a 3F° variation above the chamber temperature. With the aid of a 7-day recorder Hygro-Thermograph® and a thermometer, the temperature was kept under constant surveillance while the chamber door was open

(Figure 5). The ambient temperature of the Percival chamber was 75°F (23.9°C) ± 3F° and of the Sherer model was variable according to daily summer temperatures.

High numbers of both female and male progeny had to be maintained in the parasite colony. Thus, both fertilized female parasites, for female progeny production, and virgin female parasites, for male progeny production, were utilized during the oviposition periods.

III Parasite Growth and Development Studies

(a) Preliminary Parasite Chamber Studies

Preliminary studies were to establish the ratio of mating males to females and time required for copulation that would produce, with some surety a constant high ratio of female progeny; also to establish the length of the ovipositional period for maximum parasite progeny production, using 10 alate aphid hosts at 75°F (23.9°C) ± 3F°. On the basis of these established ratios and times, comparison studies were made keeping all factors as constant as possible except temperature. The various conditions for the preliminary studies are outlined in Table 1.

The alate greenbug form was chosen for the parasite host in the studies to give further uniformity, as Hight (1972) had indicated variations in parasite progeny sex ratios between different greenbug instars as hosts.

(b) Principal Parasite Chamber Studies

The purpose of these studies was to determine the effect of spring temperatures on the success of parasite copulation, on oviposition by virgin and fertilized females and on progeny development and emergence.

The temperature studies were done at 75°F (23.9°C) ± 3F°, 65°F (18.3°C) ± 3F°, and 55°F (12.8°C) ± 3F°. The first and third were done in the Percival model while the second was done in the Sherer chamber.

The humidistats in both chambers were set at 70%. This setting facilitated stabilization of the temperature within the cabinet (Kvenberg 1971). The Percival chamber operated with a uniformity of ± 10% but the Sherer chamber developed problems with the humidistat early in the study and the relative humidity fluctuated between 55 and 100%. The experiment was continued in this chamber as previous literature had not indicated high relative humidities a problem.

The photoperiod in the chambers was set at 15 hr from 6:00 AM to 9:00 PM, an average of the daily photoperiods over the spring months May, June, and July at Sioux Falls, South Dakota (US Navy 1959). This was the nearest location, for which photoperiod data could be obtained, to the Southeast Research Farm, Beresford, South Dakota, where further research was done on S. graminum on sorghum.

The bench height in the Percival chamber was 83 cm (32.7 inches)

from the light source whereas the bench height in the Sherer chamber was 44 cm (17.3 inches) and the floor of this chamber was 82 cm (32.3 inches) from the light source. The illumination received at pot height in the Percival model was 1350 ft-c, and in the Sherer chamber, at pot height on the bench, was 1350 ft-c, and at pot height on the chamber floor, was 310 ft-c.

Only fluorescent lighting was used, to minimize temperature variation with distance from the light source, as incandescent lighting produces radiant heat. Literature revealed that aphids can be well maintained on fluorescent lighting alone (Daniels and Jackson 1968).

Both the bench and floor space was utilized in the Sherer chamber to accomodate all 45 pots in the chamber studies.

The winter-wheat seedlings used in the chamber studies were individually grown directly in or transferred into 3-inch pots. The pots were held in 4-inch saucers. The saucers were covered with aluminum foil with a 6.4 cm (2.5 inch) central hole, reinforced with masking tape, into which the pot was placed. This minimized evaporation from the saucers when the plants were watered, stabilizing the relative humidity within the chambers (Figure 9). The plants were watered when the soil appeared dry at the surface. The necessity to water the plants decreased with each successively lower temperature level. Mold commonly formed on the soil surface at the 55°F (12.8°C) level.

The white Bristol board discs on top of the pots were numbered

from 1 to 45. The position of the pots was changed daily within the chamber according to a random numbers chart in order that each cage received an equal chance of being exposed to the same environmental conditions within the chamber. The Percival chamber had a horizontal temperature variation of 2F° and the Sherer model had both a horizontal variation and a vertical variation of 3F° as well as a variation of illumination between the bench and chamber floor levels.

From the parasite stock colony 15 newly-emerged females were individually placed, with three males of the same age, in gelatin capsules for copulation; and fifteen females remained isolated. These mated and virgin female parasites were grouped into three replicates and the respective replicate numbers were recorded on the white discs over the pots when the parasites were introduced into the cage containing biotype C greenbugs. Three replicated checks, each composed of five cages containing 10 alate, biotype C greenbugs, were included in each temperature study. As established in preliminary studies the length of the copulation period was 2 hr with a 3:1 male:female ratio and the oviposition period was 5 hr. Also, copulating groups were maintained during the oviposition period.

At the termination of the oviposition period and daily, during the period of parasite progeny development, alate forms which were not on the leaf were placed onto the leaf section with a camel's hair brush.

On the day adults started to emerge in the cages where fertilized

females had oviposited, the adults and the remaining mummies were removed from those cages into capsules. Emerged adults were removed to capsules daily from cages where virgin females had oviposited but the mummies were left. The purpose of the removal of the mummies to gelatin capsules was to observe the effect of handling at different temperature levels.

The capsules were put onto transparent Scotch® tape with a label denoting the contained mummy's origin. Those capsules containing emerged parasites were labelled with India ink according to the sex and day of emergence of the parasite. The capsules were placed in the center of the growth chamber shelf for the remainder of the experiment.

(c) Hibernation Studies

Alate mummies for the hibernation studies were developed under colony conditions. The parent parasites copulated and oviposited according to the procedures outlined for the temperature studies. The mummies were left on the leaf sections where possible and were placed individually in gelatin capsules on the 8th day after oviposition. In preliminary studies at 75°F (23.9°C) ± 3F°, emergence had been noted after the 8th day following oviposition.

Holloway (1913) had used cold storage at 38°F (3.33°C) for the transportation of immature hymenopterous egg parasites. He recommended that on removal from storage, the specimens be placed in thermos jars for about 24 hr in order for the parasites to gradually

Fig. 22.--Coleman® thermos containers used in hibernat
studies.


Coleman
Coleman
Coleman

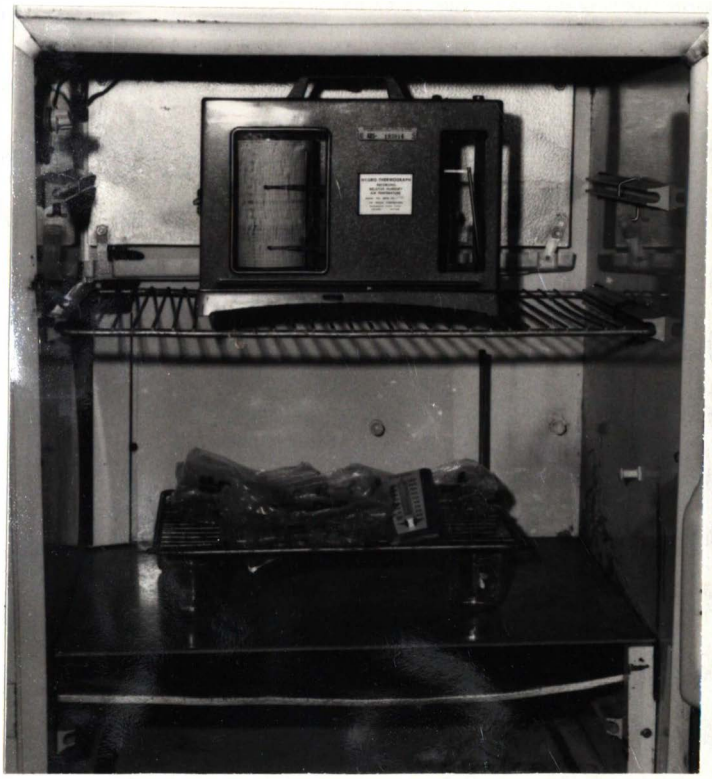
come to the temperature of the laboratory. Coleman® thermos jars were used for this purpose in the study (Figure 22).

Mummies were divided into three replicates at each of three holdover temperature levels, 24°F (-4.44°C) ± 3F°, 30°F (-1.11°C) ± 2F°, and 38°F (3.33°C) ± 2F° with relative humidities fluctuating at 50 to 100%, 60 to 100%, and 45 to 100%, respectively. In each replicate were 18 mummies, nine formed from parasitism by fertilized females and nine from parasitism by virgin females. As 100% female progeny produced by a fertilized female is less than certain, capsules containing mummies formed by the oviposition of individually mated females were spread evenly throughout the nine replicates. Each replicate was placed in labelled plastic bags, and capsules were labelled according to the probable sex of the immature parasite within (Figure 10).

Three refrigerators, manufactured by the General Air Conditioning Corporation, Los Angeles 23, California, were used for the hibernation studies, one for each temperature level (Figure 23). The internal volume of these refrigerators was approximately .8 m^3 (8.3 ft^3). The temperature was regulated by a thermostat within the refrigerator. Humidity was not regulated. A thermometer, and a Coleman® thermos jar, containing the plastic bags holding the gelatin capsules, were placed on a 27.9 X 35.6 cm (11 X 14 inches) wire rod steel shelf 26.7 cm (10.5 inches) above the floor and 5.1 cm (2.0 inches) above the freezer unit in each refrigerator.

Temperature and humidity fluctuations were monitored with a

Fig. 23.--Refrigerator unit used in hibernation studies



recording 7-day Hygro-Thermograph®, placed centrally on a wire rod steel shelf 48.2 cm (19 inches) above the floor of the refrigerator (Figure 23).

There were three sets of mummies, initially placed into the refrigerators, for hibernation periods of 1 month, 2 months, and 3 months, respectively.

The plastic bags containing the capsules were checked every 2 days for emergence during the holdover. The check intervals lasted 5 min during which time the temperatures of the chambers were raised from 2 to 4F°.

At one point the electric service at the university was shut off and the temperature of each refrigerator rose to at least 55°F (12.8°C). No emergence was noted during this 6-hr period.

Using the same procedure, one further holdover study for a period of 7 days was done, except the three replicates were sealed in thermos jars at each temperature level before being put into their respective refrigerators. The inner temperatures of the thermos jars were approximately 75°F (23.9°C). After 24 hr in the refrigerators, the capsules were removed from the jars and placed directly into the refrigerators on the bench (Figure 23). Only the temperature was checked during this holdover period.

On removal, at the termination of all hibernation studies, the capsules were checked for emergence which had occurred during the holdover and marked with India ink accordingly. The capsules were placed into thermos jars, precooled to the appropriate temperature,

which were in turn placed in a growth chamber at 75°F (23.9°C) ± 3F° and a relative humidity between 55 and 100%. After 24 hr, the capsules were removed from the thermos jars, at which time and at daily intervals thereafter, the mummies were checked for parasite emergence.

The temperature and relative humidity of the chamber were checked twice daily using a recording 7-day Hygro-Thermograph® and a thermometer.

At the end of the hibernation period the capsules were also checked for mold formation on or in the vicinity of the mummies.

3. RESULTS AND DISCUSSION

(a) Preliminary Parasite Chamber Studies

Biotype C, alate greenbug forms were used as the parasite host throughout the project. Although the biotype B colony was maintained, alate forms were not numerous enough for a comparative study with biotype C.

The preliminary parasite studies were not designed for statistical analyses but for an indication of the best combination of copulatory and ovipositional conditions.

The combination of the 3:1 parent sex ratio, with the 2 hr copulatory period, with the copulatory group maintained during oviposition, and with the 5 hr ovipositional period, was the most appropriate of the combinations examined in the preliminary studies, for maximum female progeny and overall progeny production (Table 1).

The 1:1 and 2:1 parent sex ratios produced, with varied copulatory and ovipositional periods, and with the female isolated from the males during oviposition, relatively low numbers of female progeny and resulted in low average percent emergence (Table 1). Parent sex ratios of 4:1 were attempted but resulted in a number of female parasite deaths.

The appropriate combination of factors not only gave good progeny sex ratios in favor of females and high emergence numbers, but also the 2 hr copulatory period allowed sufficient time for the experimenter to finish preparation of the parasite host material for the ovipositional period. The 5 hr ovipositional period did not

Table 1.--Sex ratios of parasite progeny from mated females, varying copulatory conditions and ovipositional conditions with 10 alate greenbug hosts at 75°F ± 3F° (23.9°C).

Parent Sex Ratio	Copulation Period (hr)	Oviposition Period (hr)	Progeny Sex Ratio: Observations 1	2	3	4	5	Avg % emergence
1:1[a]	2.75	7.25	3:0	5:1	4:0	2:0	4:0	38
1:1[b]	2.00	5.00	1:3	1:10	3:0	2:2	0:6	54
2:1[b]	1.00	4.00	1:4	3:0	1:1	1:0	2:5	36
2:1[b]	2.00	6.00	10:0	---	6:0	8:0	6:1	62
3:1[c]	1.00	4.00[e]	0:7	2:7	2:8	3:7	8:0	88
3:1[d]	2.00	5.00[e]	1:6	3:5	4:6	4:6	3:7	90

[a] Experiment date, April 16.
[b] Experiment date, April 25.
[c] Experiment date, May 4.
[d] Experiment date, May 14.
[e] Copulatory group maintained during ovipositional period.

allow greenbug honeydew accumulation nor condensation, as a result of transpiration by the contained winter wheat leaf section, to interfere with oviposition. Spencer (1926) had indicated that honeydew on the parasite could impair its functions and shorten its life.

The preliminary parasite chamber studies were done at successive intervals; and the average percent of successful adult emergence from the 10 aphid hosts increased over the last three intervals, as did the female progeny numbers. The method was the same at all intervals but it became more refined as the project advanced.

Female parasites were observed to copulate or attempt copulation several times early in the copulation period. One to three males would attempt copulation at one time within the capsules and within the Tygon(R) cages when the copulatory group was maintained. The female parasite was also noted to avoid the males when approached, and to strike at them with her ovipositor when apparently harassed.

(b) Principal Parasite Chamber Studies

The numbers of progeny produced at the three temperature levels, 75°F (23.9°C) ± 3F°, 65°F (18.3°C) ± 3F°, and 55°F (12.8°C) ± 3F°, were statistically analyzed by combining related replicates and using the Chi-square test. The offspring were divided into four groups at each temperature level: female progeny of fertilized females, male progeny of fertilized females, total progeny of fertilized females, and progeny of virgin females (Table 2). The numerical differences

Table 2.--Total emergence numbers of adult parasite progeny.

Progeny Group	Temp (°F) 75	65	55
♀[a]	87	70	37
♂[b]	28	25	14
♀ and ♂[c]	115	95	51
♂[d]	119	104	56

[a] Female progeny of mated females.
[b] Male progeny of mated females.
[c] Total progeny of mated females.
[d] Progeny of virgin females.

between emerged progeny of virgin females and between emerged progeny of mated females at the three temperature levels were highly significant (Table 3). Using the Chi-square test, recognizing that the comparisons made between progeny numbers at the three temperature levels were not independent, a significant decline in progeny numbers of both mated and virgin females was computed between the 65 and 75°F levels; and a highly significant decline, between the 55 and 75°F levels (Table 3).

Factors influencing the developing progeny at the successively lower temperatures were temperature and, as a result of greater condensation and longer periods of development, increased handling, moisture, and mold formation.

Again recognizing that the comparisons made between progeny

Table 3.--Chi-square test for emerged progeny number differences between the three temperatures.

Progeny Source					
Mated Females			Virgin Females		
Temp (°F)	df	Chi-square Value	Temp (°F)	df	Chi-square Value
All	2	58.68**	All	2	61.30**
75 vs 65	1	6.35*	75 vs 65	1	3.93*
75 vs 55	1	55.24	75 vs 55	1	54.43**

* $P < .05$.
** $P < .01$.

numbers at the three temperature levels were not independent, the Chi-square test revealed no significant difference between total progeny numbers produced by mated and virgin females at each temperature level (Table 4). The experimental method, with respect to copulation, evidently had no deleterious effect on successful oviposition by fertilized females.

Table 4.--Chi-square test for numerical differences between progeny numbers produced by mated and virgin females at each temperature level.

Temp	df	Chi-square Value
75	1	0.31
65	1	1.21
55	1	0.36

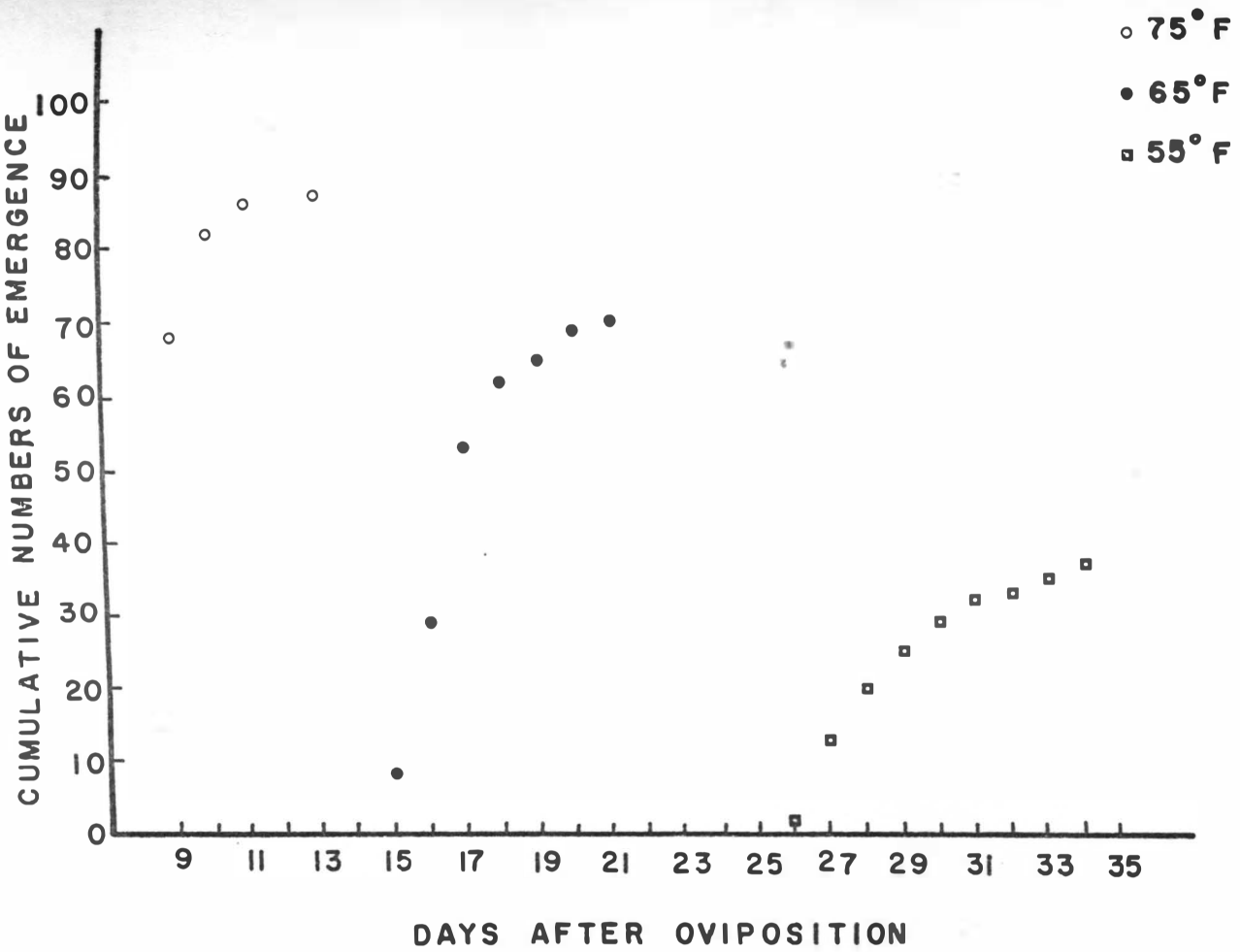
Copulation occurred at all temperature levels, although the activity among copulatory groups decreased at the successively lower temperatures. There was no significant difference between the three temperature levels with respect to progeny sex ratios of fertilized females (Table 5).

Table 5.--Chi-square test for progeny sex ratio differences between the three temperatures.

df	Chi-square Value
2	0.21

Virgin females were not observed to produce female offspring at any time during the study.

At the lower temperature levels, the rate of development and growth of female and total progeny produced by mated females decreased, as did the progeny produced by virgin females (Figures 24, 25, and 26). At 75°F, 65°F, and 55°F, the emergence of female progeny and of all the progeny of fertilized females ranged from 9 to 13, 15 to 21, and 26 to 35 days after oviposition, respectively; and the emergence of the progeny of virgin females ranged from 9 to 13, 15 to 21, and 26 to 46 days after oviposition, respectively. At 55°F, the male progeny of unmated females emerged primarily 26 to 34 days following oviposition; one emerged at 40 days; and another


75° F
65° F
55° F
CUMULATIVE NUMBERS OF EMERGENCE
0
10
20
30
40
50
60
70
80
90
100
9
11
13
15
17
19
21
23
25
27
29
31
33
35
DAYS AFTER OVIPOSITION

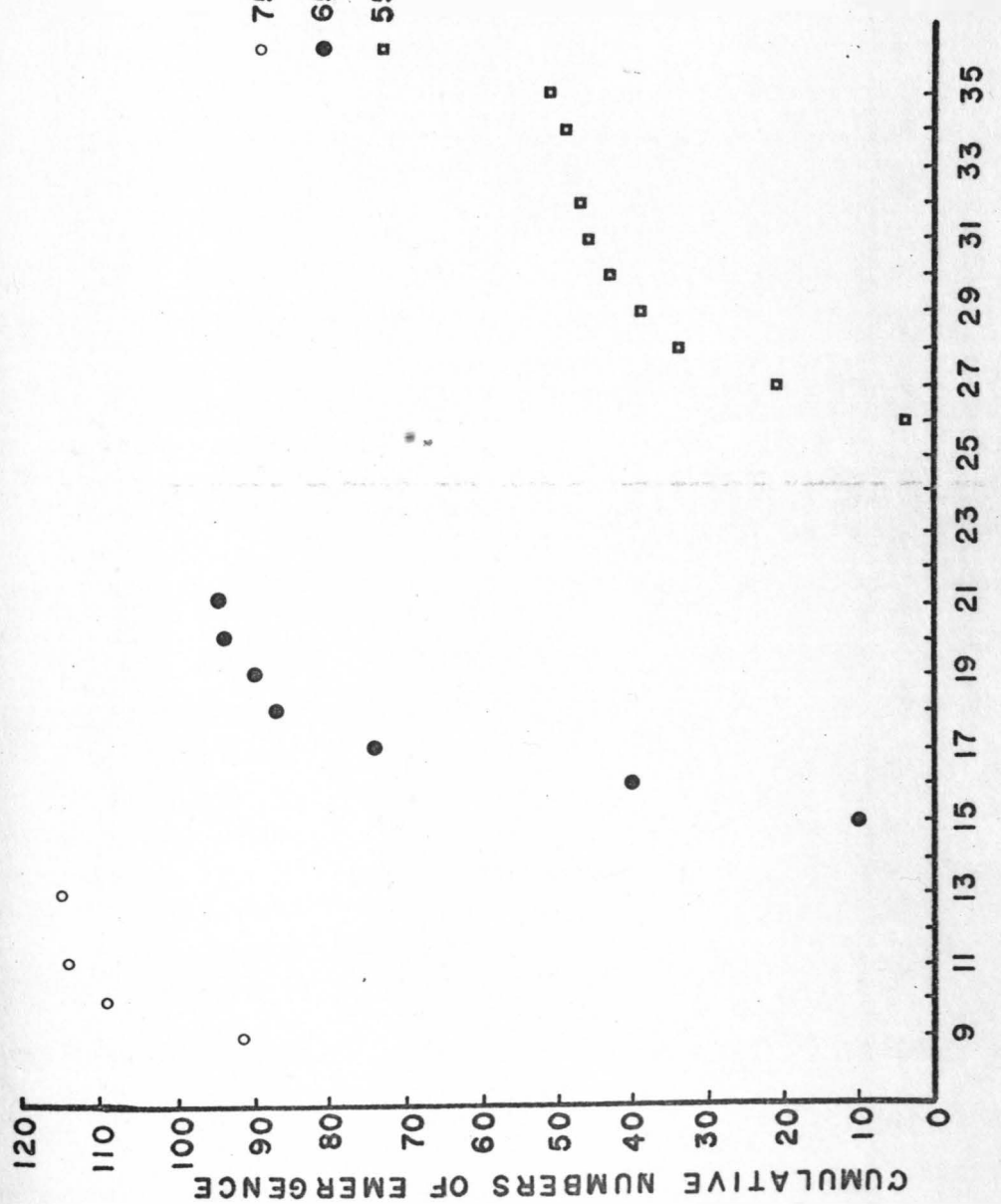
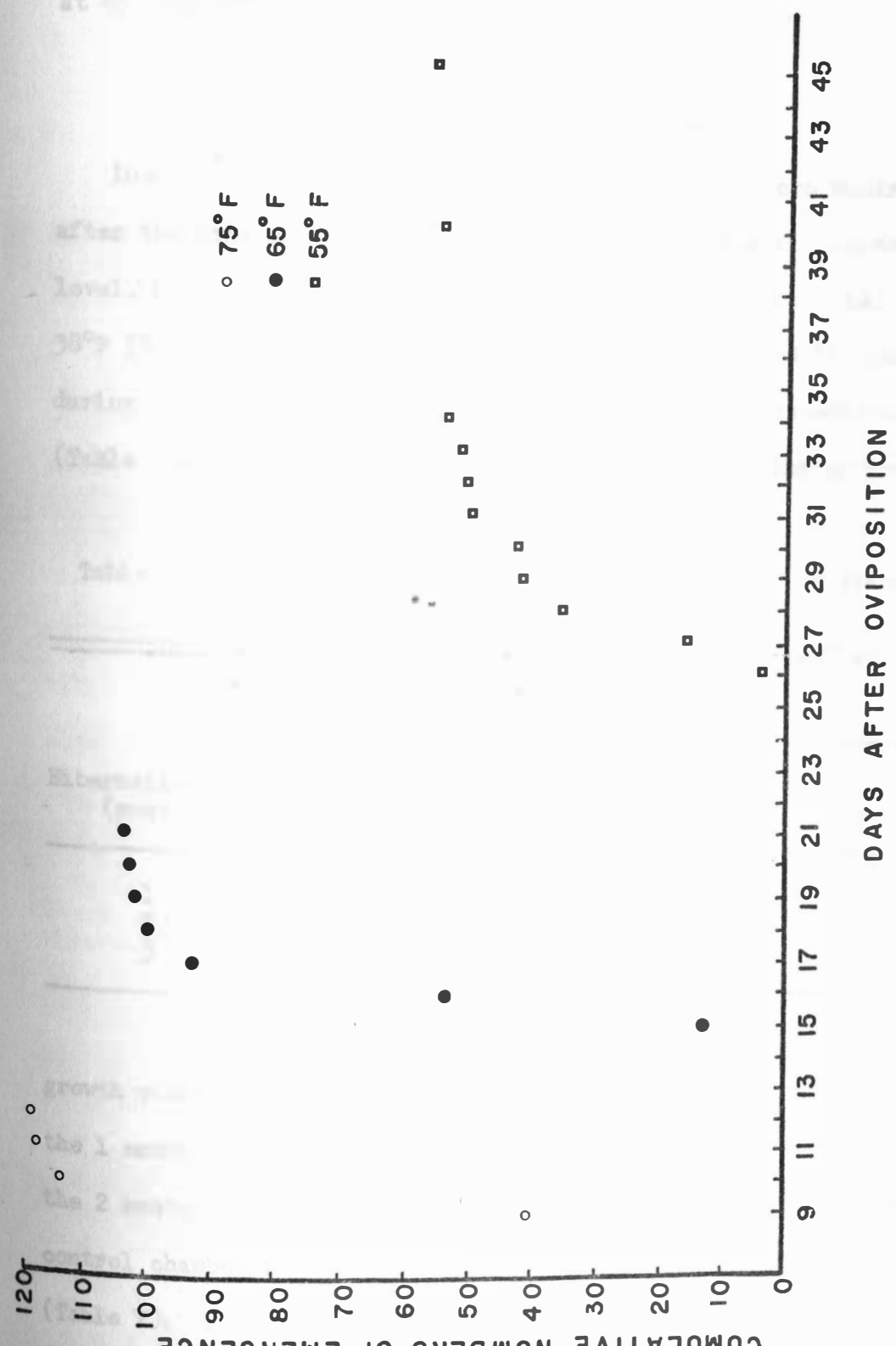

75°F
65°F
55°F
CUMULATIVE NUMBERS OF EMERGENCE
DAYS AFTER OVIPOSITION
0
10
20
30
40
50
60
70
80
90
100
110
120
9
11
13
15
17
19
21
23
25
27
29
31
33
35

Fig. 26.--Daily, accumulative, progeny emergence number virgin female parasites during the chamber studies.


75°F
65°F
55°F
CUMULATIVE NUMBERS OF EMERGENCE
0
10
20
30
40
50
60
70
80
90
100
110
120
9
11
13
15
17
19
21
23
25
27
29
31
33
35
37
39
41
43
45
DAYS AFTER OVIPOSITION

at 46 days after oviposition.

(c) Hibernation Studies

Due to the low emergence of adult parasite numbers during and after the hibernation periods, the replicates at each temperature level, for each period, were combined. Emergence was noted only at 38°F (3.33°C) ± 2F°, between 2 and 23, 20 and 21, and 18 and 33 days during the 1, 2, and 3 month hibernation periods, respectively (Table 6). Low emergence numbers were observed in the environmental

Table 6.--Total adult parasite emergence during hibernation.

Hibernation period (months)	Temp (°F)		
	38	30	24
1	8	0	0
2	2	0	0
3	3	0	0

growth chamber on the 2nd and 3rd day following the termination of the 1 month period, and on the 5th day following the termination of the 2 month period; no emergence was observed in the environmental control chamber from mummies held in hibernation for 3 months (Table 7).

Table 7.--Total adult parasite emergence after hibernation.

Hibernation period (months)	Temp (°F) 38	30	24
1	1	4	3
2	0	2	0
3	0	0	0

Using the Chi-square test, numerical differences of adult parasite emergence between the temperature levels, during each hibernation period, were computed. Differences were highly significant and significant for the 1 and 3 month hibernation periods, respectively (Table 8).

Table 8.--Chi-square values for numerical differences of emerged adult parasite progeny between temperature levels during each hibernation period.

Period (months)	df	Chi-square Value
1	2	16.83**
2	2	4.05
3	2	6.11*

* $P < .05$.
** $P < .01$.

The numerical differences of total adult parasite emergence at each temperature between the three hibernation periods, again using the Chi-square test, were significant only at the 24°F (-4.44°C) ± 3F° temperature, where emergence occurred only after the 1 month hibernation period (Table 9).

Table 9.--Chi-square values for numerical differences of the total number of adult parasites between the 1, 2, and 3 month hibernation periods at each temp.

Temp (°F)	df	Chi-square Value
38	2	2.02
30	2	4.32
24	2	6.23*

* $P < .05$.

Differences, in the emerged adult progeny numbers between the temperature levels for the 1 and 2 month hibernation periods, after removal from the refrigerators to the environmental growth chamber, were computed to be non-significant using the Chi-square test (Table 10).

The data of the 1 week hibernation study was analyzed separately from the data of the 1, 2, and 3 month hibernation periods, as the adult parasites which had emerged under refrigeration conditions were active during the period when the remaining parasites emerged in the

Table 10.--Chi-square values for numerical differences of emerged adult parasite progeny between temperature levels after each hibernation period.

Period (month)	df	Chi-square Value
1	2	1.84
2	2	4.05

environmental growth chamber. The progeny were divided into three groups for the Chi-square test: total progeny of mated females, female progeny of mated females, and progeny of virgin females (Table 11). No significant differences were noted between

Table 11.--Total adult parasite emergence from mummies at each hibernation temperature after 1 week of hibernation.

	Parent				
	Mated			Virgin	
Temp (°F)	Mummies[a]	♀and♂[b]	♀[c]	Mummies[d]	♂[e]
38	24	21	17	22	17
30	24	21	17	21	17
24	24	21	16	22	19

[a] Mummies containing potential females.
[b] Total progeny, emerged from mummies produced by mated females.
[c] Female progeny, emerged from mummies produced by mated females.
[d] Mummies containing potential males.
[e] Male progeny, emerged from mummies produced by virgin females.

temperature levels for any group (Table 12).

Table 12.--Chi-square values for numerical differences of emerged adult parasite progeny between hibernation temp levels, 38, 30, and 24°F, for the 3 progeny groups after a 1 week period of hibernation.

Progeny group	df	Chi-square Value
♀[a]	2	0.00
♀ and ♂[b]	2	0.13
♂[c]	2	0.61

[a] Female progeny of mated females.
[b] Total progeny of mated females.
[c] Progeny of virgin females.

Mold was not observed on the mummies after the 1 week hibernation period but was observed on 26%, 48%, and 27% of the mummies held at 1, 2, and 3 months, respectively.

The 1 week and the 1 month hibernation periods unlike the 2 or 3 month periods were not subject to the electricity failure and the subsequent temperature fluctuation.

IV POPULATION STUDIES ON SYSTEMIC INSECTICIDES TREATED GRAIN SORGHUM (FIELD)

1. MATERIALS AND METHODS

(a) Planting Treatments and Application

Field research was carried on at the Southeast Research Farm, Beresford, South Dakota during the summer of 1972 using the Northrup King 222 variety of sorghum. The planting date was June 5. The row spacing was 30 inches and a John Deere® Planter was used.

The seed-bed preparation involved fall plowing. Ramrod®, 65w, was the herbicide, applied as an overall spray at 6.5 lb/acre. Fertilizer, consisting of 100 lb ammonium nitrate (nitrogen), 35 lb superphosphate (P_2O_5), and 30 lb muriate potash (K_2O)/acre, was broadcast and disced in before planting. The plant population was 50,000 plants/acre.

The field area was divided into five replicates, with 2.5 replicates on either side of untreated sorghum buffer rows. At each end of the replicate groups was a buffer area of 8 rows of sorghum. A list of the insecticides used and their rates are in Table 13. The insecticides were primarily applies in a 4- to 7-inch band, but Furadan® was applied in the furrow with the seed in two treatments and Orthene® was applied as a seed treatment as well as in a band. The dimensions of each replicate were 65 ft X 140 ft except for replicate five which was divided into two equal areas of which the dimensions were 65 ft X 70 ft.

Table 13.--Systemic insecticides applied in band at sorghum planting time, June 5, 1972, at Southeast Research Farm, Beresford, South Dakota.

Treatment No.	Trade Name	Common Name	Rates (lb AI/acre)	Chemical Name
1, 2, 3	Orthene 10G	acephate	1.0, 0.5, 0.3[a]	O,S-Dimethyl acetyl-phosphoramido thioate $C_4H_{10}NO_3PS$
4, 12	Di-Syston 15G	disulfoton, thiodemeton	1.0, 0.5	O,O-Diethyl S-(2-(ethylthio) ethyl) phosphorodi-thioate
5, 6, 7	Furadan 10G	carbofuran	0.5[c], 1.0[c], 1.0	2,3-Dihydro-2, 2-dimethyl 7-benzofuranyl methyl-carbamate
8	C.G.A. 12658 5G[b]	———	1.0	unavailable classified information[d]
9, 10	A.C. 92100 15G[b]	———	1.0, 0.5	unavailable classified information
11	Temik 10G	aldicarb	1.0	2-Methyl-2 (methylthio) propion-aldehyde O-(methyl carbamayl) oxime

[a] Applied as a seed treatment, lb AI/100 lb seed.
[b] Unregistered.
[c] Applied in band with seed.
[d] ENT. no. 27,920.

The insecticide treatments and one check were arranged randomly within each replicate.

(b) Evaluation

A count involving only greenbugs was made at 31 days after planting. The first greenbug count was taken from entire plants and the second and third greenbug counts were taken from a third level leaf where greenbug populations appeared the most dense. Additional counts of predators and mummies, were taken at 37 and 58 days after planting on the same third leaf level as the greenbug counts. Further predator counts were taken from the whole plants. All counts were taken from five plants at five-pace intervals from the two center rows in each treatment and check.

Counts were made of Coccinellidae, adults and larvae; Chrysopidae and Hemerobiidae, adults and larva; Syrphidae, adults and larvae; parasite adults, and parasite mummies.

Parasites collected the summer of 1972 were sent to the Plant Pest Survey and Detection Division of the United States Department of Agriculture for identification.

2. RESULTS AND DISCUSSION

Greenbugs were first noted on the sorghum seedlings, at the Southeast South Dakota Experiment Farm, 13 days after planting.

Least-squares analysis of variance indicated significant differences between greenbug populations at 37 days after planting, and between parasite mummy populations at 37 and 58 days after planting, either among the treatments and the untreated checks or among the treatments alone. No statistically significant differences were noted between greenbug populations at 31 and 58 days after planting, nor between Coccinellidae, adult and larval, populations at 37 and 58 days after planting. Population counts of Syrphidae, adults and larvae; of Chrysopidae and Hemerobiidae, adults and larvae; and of adult parasitic hymenoptera were too low for statistical analysis.

Dunnet's values were derived for the data, which the analysis of variance had indicated to have significant differences (Table 14).

Table 14.--Dunnet's value for comparing treatments to check where the least-squares analysis of variance indicated treatment significance between insect populations.

Population	Days after Treatment	Dunnet's Value P<.05	Dunnet's Value P<.01
Greenbug	37	23.91	29.10
Parasite mummies	37	0.56	0.90
Parasite mummies	58	63.37	77.14

The greenbug populations on treated sorghum, 37 days after planting, were not significantly different from those populations on untreated checks but were significantly different among the treatments. Treatments 6, 9, and 11 had the lowest greenbug populations. There were also significant differences in parasite mummy populations among treatments, 58 days after planting. The parasite mummy populations, 37 days after planting, on treated grain sorghum were significantly lower than the untreated check populations in treatments 5, 7, 8, and 9 at the .05 level of probability and in treatments 6 and 11 at the .01 level (Table 15).

The significantly lower parasite mummy numbers were not necessarily reflective of action against the immature or mature parent parasite but could have been an indication of the unsuitability of the systemically treated host for the greenbug.

Although differences in greenbug populations were not statistically significant at 31 days after planting, on comparing the least-squares means of the populations, certain values might be considered biologically significant. The least-squares means for the greenbug populations, 58 days after planting, and the adult and larval Coccinellidae populations, 37 and 58 days after planting, were similar.

The hyperparasite, Pachyneuron siphonophorae (Ashmead) in the family Pteromalidae was collected from South Dakota sorghum fields in 1971 (Figure 27). Tentative identifications were made for

Table 15.--Least-squares means of insect populations for which the least-squares analysis of variance indicated treatment significance.

Treatment	Least-squares Means		
	Greenbug 37 days	Parasite mummies 37 days	Parasite mummies 58 days
Untreated	20.76	1.76	43.60
1	30.00	1.43	53.72
2	27.48	2.04	76.08
3	32.48	1.85	56.84
4	14.40	1.53	26.52
5	25.12	1.11*	44.20
6	4.40	0.78**	4.28
7	13.60	0.97*	45.92
8	11.16	0.95*	25.36
9	6.80	1.10*	6.28
10	20.72	1.48	53.96
11	0.32	0.73**	0.88
12	27.32	1.92	80.76

* $P < .05$.
** $P < .01$.

L. testaceipes and P. siphonophorae, collected from South Dakota sorghum fields in 1972. These identifications are not, as yet, verified.

V CONCLUSIONS

The 3:1 parent sex ratio of L. testaceipes with a 2 hr copulation and a 5 hr ovipositional period, with the copulatory group maintained during the ovipositional period, produced a relatively high number of female progeny accompanied by relatively high total emergence numbers at 75°F (23.9°C) ± 3F°.

As the temperature declined from 75°F (23.9°C) ± 3F° to 55°F (12.8°C) ± 3F°, the numbers of emerged progeny of both virgin and female parasites declined significantly. The decrease in progeny numbers, statistically was highly significant between 75°F (23.9°C) ± 3F° and 55°F (12.8°C) ± 3F° and significant between 75°F (23.9°C) ± 3F° and 65°F (17.2°C) ± 3F°. As the numbers of emerged progeny of mated and virgin females were similar at each temperature, the variations in conditions had affected equally the mated and virgin females oviposition and the development of growth of their progeny. The declining temperatures caused a resultant decrease in activity but not sufficient, under the experimental procedure of this project, to cause a statistically significant decline in the progeny sex ratio of mated females.

The development and growth period for L. testaceipes increased at each successively lower temperature level, 75°F (23.9°C) ± 3F°, 65°F (18.3°C) ± 3F°, and 55°F (12.8°C) ± 3F°, such that there was no overlapping of emergence periods. Also, at successively lower temperatures the range of the emergence period increased and at lower levels accentuated an innate capacity of the species to stagger the

time of emergence. At 55°F the male emergence period exceeded that of the female by 11 days.

Low adult emergence numbers occurred during and after hibernation. Adult emergence at 38°F (3.33°C) ± 2F° occurred primarily during the hibernation periods with no emergence at the termination of the 2 and 3 month periods. The 30°F (-1.11°C) ± 2F° and the 24°F (-4.44°C) ± 3F° temperatures were sufficiently low to inhibit emergence during the hibernation period. At the 30°F temperature statistically non-significant differences in emergence numbers were noted between hibernation periods. At the 24°F temperature, while emergence occurred at the termination of the 1 month period, hibernation periods of 2 and 3 months were not conducive to immature parasite survival.

The 1 month hibernation period at 30°F, with the experimental conditions described, appeared to be the most appropriate hibernation period and temperature for holdover of immature parasites. There was no emergence during the hibernation period and emergence numbers were highest at the 30°F at the termination of the period, although not significantly different from the emergence numbers at 24°F.

After removal from the refrigerators, there were no significant differences in the adult parasite emergence numbers between the temperature levels of each hibernation period.

High and equitable adult parasite emergence numbers were observed at all hibernation temperatures after the 1-week period of refrigeration; and there was no significant differences in the emergence numbers between the temperatures.

The statistically analyzed data from the field treatments gave varied results. At 37 days after planting, although treatment greenbug populations did not differ significantly from the untreated check populations, there were highly significant differences among treatment populations. This indicated that the check counts fell into the mid-range of the treatment counts. The lowest greenbug numbers were on sorghum treated with Furadan(R) 10G, applied in a sub-surface band with the seed at 1.0 lb AI/acre, and A.C. 92100 15G, and Temik(R) 10G, applied in a band over the row at 1.0 lb AI/acre. These treatments produced relatively low parasite mummy populations at 58 days after planting. At 37 days after planting, parasite mummy populations differed from the checks at the .05 level of probability on sorghum treated with Furadan(R) 10G, applied in a sub-surface band with the seed at 0.5 lb AI/acre and in a band over the row at 1.0 lb AI/acre; and with C.G.A. 12558 5G and A.C. 92100 15G, applied in a band over the row at the 1.0 lb AI/acre. Parasite mummy populations differed from the checks at the .01 level of probability on sorghum treated with Furadan(R) 10G, applied in a sub-surface band with the seed at 1.0 lb AI/acre, and Temik(R) 10G, applied in a band over the row at 1.0 lb AI/acre.

The author recognizes that the laboratory results were not necessarily reflective of the field parasite populations, but these observations, with the field observations on the effect of systemic insecticides on the parasite-greenbug complex, contribute further information towards the eventual integrated control of the greenbug.

Similar studies could be done, omitting the high humidities at successively lower temperatures and thus alleviating the problems of handling and mold. The lower temperature limit for successful copulation and oviposition was not established in this project.

Future work on the hibernation of L. testaceipes, in the laboratory alleviating the problems of moisture and mold, may result in higher emergence numbers.

Further studies should be pursued with respect to the relationship between L. testaceipes and P. siphonophorae as the latter was observed frequently during the sorghum growing season. Also, investigations should be made as to the economically appropriate time of mass release of L. testaceipes into the field.

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VIII APPENDIX

Table 16.--Emergence of female parasite progeny, produced by fertilized females at 75°F (23.9°C) ± 3F°, days after oviposition.

Day	Replicate 1		Replicate 2		Replicate 3	
	Daily	Accumulated	Daily	Accumulated	Daily	Accumulated
9	17	17	24	24	27	27
10	8	25	3	27	3	30
11	3	28*	1	28	0	30
12	0	28	0	28	0	30
13	0	28	1	29	0	30

* Emergence ceased.

Table 17.--Emergence of total parasite progeny, produced by fertilized females at 75°F (23.9°C) ± 3F°, days after oviposition.

Day	Replicate 1		Replicate 2		Replicate 3	
	Daily	Accumulated	Daily	Accumulated	Daily	Accumulated
9	28	28	29	29	34	34*
10	10	38	3	32	5	39*
11	4	42*	1	33	0	39
12	0	42	0	33	0	39
13	0	42	1	34	0	39

* Emergence ceased.

Table 18.--Emergence of male parasite progeny, produced by virgin females at 75°F (23.9°C) ± 3F°, days after oviposition.

	Replicate 1		Replicate 2		Replicate 3	
Day	Daily	Accumulated	Daily	Accumulated	Daily	Accumulated
9	24	24	16	16	1	1
10	20	44	26	42	27	28
11	0	44	1	43*	3	31*
12	1	45*	0	43	0	31
13	0	45	0	43	0	31

* Emergence ceased.

Table 19.--Emergence of female parasite progeny, produced by fertilized females at 65°F (18.3°C) ± 3F°, days after oviposition.

Day	Replicate 1		Replicate 2		Replicate 3	
	Daily	Accumulated	Daily	Accumulated	Daily	Accumulated
15	3	3	4	4	1	1
16	4	7	9	13	8	9
17	1	8	3	16	20	29
18	3	11	4	20	2	31
19	1	12	1	21	1	32
20	1	13*	2	23	1	33*
21	0	13	1	24	0	33

* Emergence ceased.

Table 20.--Emergence of total parasite progeny, produced by fertilized females at 65°F (18.3°C) ± 3F°, days after oviposition.

Day	Replicate 1		Replicate 2		Replicate 3	
	Daily	Accumulated	Daily	Accumulated	Daily	Accumulated
15	3	3	6	6	1	1
16	11	14	9	15	10	11
17	11	25	3	18	20	31
18	4	29	7	25	2	33
19	1	30	1	26	1	34
20	1	31*	2	28	1	35*
21	0	31	1	29	0	35

* Emergence ceased.

Table 21.--Emergence of male parasite progeny, produced by virgin females at 65°F (18.3°C) ± 3F°, days after oviposition.

Day	Replicate 1		Replicate 2		Replicate 3	
	Daily	Accumulated	Daily	Accumulated	Daily	Accumulated
15	6	6	7	7	0	0
16	19	25	11	18	11	11
17	13	38	11	29	15	26
18	1	39	5	34	1	27
19	1	40*	0	34	1	28*
20	0	40	1	35	0	28
21	0	40	1	36	0	28

* Emergence ceased.

Table 22.--Emergence of female parasite progeny, produced by fertilized females at 55°F (12.8°C) ± 3F°, days after oviposition.

Day	Replicate 1		Replicate 2		Replicate 3	
	Daily	Accumulated	Daily	Accumulated	Daily	Accumulated
26	1	1	1	1	0	0
27	5	6	6	7	0	0
28	1	7	5	12	1	1
29	2	9	2	14	1	2
30	0	9	0	0	4	6*
31	2	11	1	15	0	6
32	1	12*	0	0	0	6
33	0	12	0	0	0	6
34	0	12	2	17	0	6
35	0	12	2	19*	0	6

* Emergence ceased.

Table 23.--Emergence of total parasite progeny, produced by fertilized females at 55°F (12.8°C) ± 3F°, days after oviposition.

Day	Replicate 1		Replicate 2		Replicate 3	
	Daily	Accumulated	Daily	Accumulated	Daily	Accumulated
26	1	1	1	1	2	2
27	9	10	7	8	1	3
28	2	12	5	13	6	9
29	2	14	2	15	1	10
30	0	14	0	15	4	14
31	2	16	1	16	0	14*
32	1	17*	0	16	0	14
33	0	17	0	16	0	14
34	0	17	2	18	0	14
35	0	17	2	20	0	14

* Emergence ceased.

Table 24.--Emergence of male parasite progeny, produced by virgin females at 55°F (12.8°C) ± 3F°, days after oviposition.

Day	Replicate 1		Replicate 2		Replicate 3	
	Daily	Accumulated	Daily	Accumulated	Daily	Accumulated
26	3	3	1	1	7	7
27	3	6	2	3	7	14
28	4	10	9	12	1	15
29	2	12	3	15	0	15
30	1	13	0	15	2	17
31	2	15	3	18	1	18
32	0	15	0	18	0	18
33	0	15	1	19	1	19*
34	1	16*	0	19	0	19
40	0	16	1	20	0	19
46	0	16	1	21	0	19

* Emergence ceased.

Table 25.--Adult parasite emergence during three hibernation periods from mummies[a] held at three refrigeration temperatures, 8 days following oviposition at 75°F (23.9°C) ± 3F°.

Hibernation Period (month)	Day[b]	Temp (°F) 38 ♂	38 ♀	30 ♂	30 ♀	24 ♂	24 ♀
1	2	0	1	0	0	0	0
	3	1	0	0	0	0	0
	4	0	0	0	0	0	0
	5	0	1	0	0	0	0
	9	1	1	0	0	0	0
	16	1	0	0	0	0	0
	19	0	1	0	0	0	0
	23	0	1	0	0	0	0
2	20	0	1	0	0	0	0
	21	1	0	0	0	0	0
3	18	0	1	0	0	0	0
	27	1	0	0	0	0	0
	33	1	0	0	0	0	0

[a] Groups of mummies consisting of nine potential ♀ adults and nine potential ♂ adults were placed at each temperature level for each hibernation period.
[b] Days following initiation of hibernation.

Table 26.--Adult parasite emergence, from groups of mummies held at different refrigeration temperatures and for different hibernation periods, after removal from the refrigerators to an environmental growth chamber, with a temperature at 75°F (23.9°C) ± 3F°.

Hibernation Period (month)	Day[a]	Temp (°F) 38 ♂	38 ♀	30 ♂	30 ♀	24 ♂	24 ♀
1	2	0	1	1	1	0	0
	3	0	0	1	1	2	1
2	5	0	0	0	2	0	0

[a] Days after removal from refrigerators.

Table 27.--Total adult parasite emergence numbers, from mummies held in refrigerators at different temperatures for 1 week, after removal to an environmental growth chamber with temperature at 75°F (23.9°C) ± 3F°.

		Progeny Source		
		Mated [a]		Virgin [b]
Temp (°F)	Day[c]	♀ and ♂[d]	♀[e]	♂[f]
38	0[g]	0	0	0
	1	19	15	17
	2	1	1	0
	3	1	1	0
30	0[g]	1	1	3
	1	14	11	13
	2	6	5	1
	3	0	0	0
24	0[g]	0	0	1
	1	13	11	15
	2	7	4	3
	3	1	1	0

[a] Number of emerged progeny from 24 mummies.
[b] Number of emerged progeny from 22 mummies, 38 and 24°F, and 21 mummies, 30°F.
[c] Day of emergence from date of removal from refrigerators.
[d] Total number of emerged progeny from the mated female source.
[e] Number of emerged female progeny from the mated female source.
[f] Number of emerged male progeny from the virgin female source.
[g] Number of progeny which emerged during refrigeration.

Table 28.--Greenbug field population counts per sorghum plant, 31 days after planting, replicate 1.

Treatment	Observation				
	1	2	3	4	5
1	15	9	20	5	6
2	14	22	0	48	62
3	2	11	7	37	22
4	1	20	11	11	7
5	14	6	21	17	1
6	0	0	0	0	0
7	2	1	4	4	5
8	0	0	1	11	1
9	0	380	33	18	3
10	26	24	43	15	20
11	0	1	0	1	1
12	9	28	25	35	0
Untreated Check	22	20	18	18	15

Table 29.--Greenbug field population counts per sorghum plant, 31 days after planting, replicate 2.

	Observation				
Treatment	1	2	3	4	5
1	110	73	33	28	63
2	21	11	11	63	61
3	43	59	16	5	50
4	15	33	3	32	12
5	26	22	21	15	21
6	18	0	32	30	18
7	53	108	16	26	14
8	38	66	50	15	0
9	3	1	20	2	7
10	157	25	38	78	26
11	1	2	0	0	0
12	23	62	15	37	70
Untreated Check	26	17	34	26	18

Table 30.--Greenbug field population counts per sorghum plant, 31 days after planting, replicate 3.

Treatment	Observation				
	1	2	3	4	5
1	19	14	21	0	2
2	18	17	51	8	3
3	7	23	9	6	5
4	7	4	3	3	12
5	1	5	18	1	10
6	0	1	1	3	0
7	6	7	0	45	27
8	7	0	3	8	2
9	1	12	1	1	2
10	0	2	2	14	17
11	1	0	1	4	1
12	22	8	9	21	20
Untreated Check	26	6	45	7	9

Table 31.--Greenbug field population counts per sorghum plant, 31 days after planting, replicate 4.

Treatment	Observation 1	2	3	4	5
1	31	35	17	20	20
2	38	9	31	22	34
3	5	5	30	20	60
4	10	37	22	8	4
5	26	30	18	0	6
6	1	1	0	0	0
7	19	21	0	6	9
8	15	9	6	6	19
9	12	35	0	5	0
10	36	1	0	4	46
11	3	3	2	0	2
12	33	3	39	5	9
Untreated Check	8	7	0	55	20

Table 32.--Greenbug field population counts per sorghum plant, 31 days after planting, replicate 5.

Treatment	Observation				
	1	2	3	4	5
1	7	1	12	8	1
2	2	20	0	34	6
3	15	20	4	22	12
4	16	44	25	9	1
5	4	2	37	12	17
6	22	24	27	12	9
7	1	5	13	23	24
8	0	2	6	4	0
9	1	0	10	0	13
10	9	16	22	27	14
11	1	0	0	2	1
12	10	49	34	2	36
Untreated Check	11	8	10	9	0

Table 33.--Greenbug field population counts from a single leaf at the third sorghum leaf level, 37 days after planting, replicate 1.

Treatment	Observation				
	1	2	3	4	5
1	1	65	3	1	1
2	33	2	3	0	0
3	0	25	0	27	4
4	14	5	1	10	5
5	13	17	32	0	9
6	0	0	0	0	0
7	0	9	0	2	2
8	0	0	6	0	3
9	40	2	18	2	17
10	32	45	8	14	16
11	0	0	0	7	0
12	10	18	30	5	3
Untreated Check	22	65	45	17	11

Table 34.--Greenbug field population counts from a single leaf at the third sorghum leaf level, 37 days after planting, replicate 2.

Treatment	Observation				
	1	2	3	4	5
1	55	60	35	85	30
2	15	100	25	0	120
3	90	15	35	15	5
4	15	30	30	15	5
5	30	5	135	25	50
6	8	1	2	15	1
7	10	4	85	20	15
8	60	55	45	10	1
9	2	0	0	0	4
10	20	35	40	70	5
11	0	0	0	0	0
12	5	60	90	10	5
Untreated Check	10	20	25	5	0

Table 35.--Greenbug field population counts from a single leaf at the third sorghum leaf level, 37 days after planting, replicate 3.

Treatment	Observation				
	1	2	3	4	5
1	4	43	5	45	7
2	7	35	23	9	0
3	23	25	0	10	0
4	0	0	0	0	9
5	72	3	3	0	0
6	0	6	1	0	0
7	25	3	5	10	0
8	0	5	9	7	3
9	0	0	0	11	12
10	1	0	6	1	0
11	0	0	0	0	0
12	0	35	40	32	0
Untreated Check	42	0	29	9	1

Table 36.--Greenbug field population counts from a single leaf at the third sorghum leaf level, 37 days after planting, replicate 4.

Treatment	Observation				
	1	2	3	4	5
1	110	30	10	3	2
2	20	45	15	15	25
3	160	15	160	60	50
4	2	0	30	20	1
5	20	8	15	25	130
6	0	0	0	6	0
7	33	10	5	20	15
8	1	0	3	20	15
9	20	10	1	10	0
10	10	1	15	10	145
11	0	0	1	0	0
12	65	35	35	7	12
Untreated Check	1	25	15	10	15

Table 37.--Greenbug field population counts from a single leaf at the third sorghum leaf level, 37 days after planting, replicate 5.

Treatment	Observation				
	1	2	3	4	5
1	130	15	5	0	5
2	20	35	15	80	45
3	40	35	3	15	0
4	42	13	76	16	21
5	3	15	7	1	10
6	0	45	10	3	12
7	1	1	5	40	20
8	0	1	15	10	10
9	0	3	10	3	5
10	6	4	7	7	20
11	0	0	0	0	0
12	4	32	30	95	25
Untreated Check	5	82	5	41	9

Table 38.--Greenbug field population counts from a single leaf at the third sorghum leaf level, 58 days after planting, replicate 1.

Treatment	Observation				
	1	2	3	4	5
1	1	1	48	0	1
2	0	2	2	0	1
3	1	0	4	4	14
4	0	0	0	0	0
5	0	0	20	1	20
6	0	0	0	0	0
7	0	0	6	0	15
8	0	5	5	3	15
9	0	2	0	0	0
10	0	0	0	0	5
11	0	0	0	0	0
12	0	5	0	0	1
Untreated check	0	0	0	0	0

Table 39.--Greenbug field population counts from a single leaf at the third sorghum leaf level, 58 days after planting, replicate 2.

Treatment	Observation				
	1	2	3	4	5
1	0	2	35	1	5
2	0	35	3	4	0
3	0	0	0	1	7
4	0	12	0	0	0
5	0	10	0	15	0
6	2	0	0	40	1
7	0	1	5	0	0
8	0	2	1	0	0
9	0	0	0	0	2
10	0	1	2	1	5
11	0	0	0	0	0
12	0	0	0	0	12
Untreated Check	9	0	1	10	0

Table 40.--Greenbug field population counts from a single leaf at the third sorghum leaf level, 58 days after planting, replicate 3.

Treatment	Observation				
	1	2	3	4	5
1	0	0	0	0	0
2	9	3	0	3	0
3	1	0	0	3	0
4	0	0	0	0	0
5	0	0	0	2	0
6	0	10	1	1	0
7	0	0	0	0	3
8	0	0	0	0	0
9	0	4	0	0	0
10	4	41	8	11	0
11	0	0	0	0	0
12	0	0	0	0	0
Untreated Check	0	3	0	0	0

Table 41.--Greenbug field population counts from a single leaf at the third sorghum leaf level, 58 days after planting, replicate 4.

Treatment	Observation				
	1	2	3	4	5
1	0	0	3	0	0
2	10	8	2	1	0
3	2	0	0	0	0
4	0	0	0	0	0
5	0	0	0	0	0
6	1	0	0	0	0
7	0	0	4	0	1
8	8	0	7	0	0
9	0	0	0	0	1
10	1	8	1	0	5
11	0	0	13	0	0
12	0	0	1	0	11
Untreated Check	0	1	9	0	4

Table 42.--Greenbug field population counts from a single leaf at the third sorghum leaf level, 58 days after planting, replicate 5.

Treatment	Observation				
	1	2	3	4	5
1	7	8	3	4	0
2	0	5	1	11	0
3	0	0	0	5	2
4	0	0	0	0	6
5	0	10	8	5	1
6	0	7	0	0	7
7	18	23	0	1	2
8	0	2	1	3	8
9	0	0	0	0	0
10	0	0	0	0	15
11	0	0	0	0	0
12	0	1	0	0	0
Untreated Check	0	0	0	0	0

Table 43.--Coccinellidae, adult (A) and larval (L), field population counts per sorghum plant, 37 days after planting, replicate 1.

Treatment	Observation									
	1		2		3		4		5	
	A	L	A	L	A	L	A	L	A	L
1	0	0	0	0	0	0	1	0	0	0
2	0	0	1	0	0	0	0	0	0	0
3	0	0	0	0	0	0	1	0	0	0
4	0	0	0	0	0	0	1	0	1	0
5	0	0	1	0	0	0	0	0	0	0
6	0	0	0	0	0	0	0	0	0	0
7	0	0	0	0	1	0	1	0	0	0
8	0	0	0	0	0	0	1	0	0	0
9	0	0	0	0	0	0	0	0	1	0
10	0	0	1	0	0	0	0	0	1	0
11	0	0	0	0	0	0	0	0	0	1
12	1	0	0	0	0	0	2	0	0	0
Untreated Check	0	0	0	0	0	0	0	0	0	0

Table 44.--Coccinellidae, adult (A) and larval (L), field population counts per sorghum plant, 37 days after planting, replicate 2.

Treatment	Observation									
	1		2		3		4		5	
	A	L	A	L	A	L	A	L	A	L
1	0	0	0	0	1	0	1	0	0	0
2	0	0	1	0	0	1	0	0	0	0
3	0	0	1	0	1	0	0	0	0	0
4	1	0	0	0	0	0	0	0	0	0
5	0	0	1	0	0	0	0	0	0	0
6	0	0	0	0	0	0	0	0	1	0
7	0	0	0	0	0	0	0	0	0	0
8	1	0	0	0	0	0	0	0	0	0
9	0	0	0	0	0	0	0	0	0	0
10	0	0	0	0	0	0	0	0	1	0
11	0	0	0	0	0	0	0	0	0	0
12	0	0	0	0	0	0	0	0	0	16
Untreated Check	1	0	0	0	0	0	0	0	0	2

Table 45.--Coccinellidae, adult (A) and larval (L), field population counts per sorghum plant, 37 days after planting, replicate 3.

Treatment	Observation									
	1		2		3		4		5	
	A	L	A	L	A	L	A	L	A	L
1	0	0	0	16	0	0	0	2	0	0
2	0	14	0	0	0	0	0	0	1	0
3	0	0	0	0	1	0	0	0	0	0
4	0	0	0	0	0	0	0	0	0	0
5	0	1	0	0	0	0	0	0	0	0
6	0	0	0	0	0	0	0	0	0	0
7	0	0	0	0	0	0	0	0	0	0
8	0	0	0	0	0	0	1	0	0	0
9	0	0	1	0	0	0	0	0	0	0
10	0	0	0	0	0	0	1	0	0	0
11	1	0	0	0	0	0	0	6	0	0
12	1	0	0	0	0	0	1	0	1	0
Untreated Check	1	0	0	0	0	0	1	0	0	0

Table 46.--Coccinellidae, adult (A) and larval (L), field population counts per sorghum plant, 37 days after planting, replicate 4.

Treatment	Observation									
	1		2		3		4		5	
	A	L	A	L	A	L	A	L	A	L
1	0	0	0	0	0	0	0	0	0	17
2	0	0	1	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0
4	0	0	0	0	0	0	0	0	0	0
5	1	0	0	0	0	0	0	0	0	0
6	0	0	0	0	0	0	0	0	1	0
7	0	0	0	0	0	0	0	0	0	0
8	0	0	0	0	0	0	0	0	0	0
9	0	0	0	0	0	0	0	0	0	0
10	0	0	0	0	0	0	0	0	0	0
11	0	0	0	0	0	0	0	0	0	0
12	0	0	0	0	0	0	0	7	0	0
Untreated Check	1	0	0	0	0	0	0	0	0	0

Table 47.--Coccinellidae, adult (A) and larval (L), field population counts per sorghum plant, 37 days after planting, replicate 5.

Treatment	Observation									
	1		2		3		4		5	
	A	L	A	L	A	L	A	L	A	L
1	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0
4	1	0	1	0	0	0	0	0	0	0
5	0	0	0	0	0	0	0	0	0	0
6	0	0	0	0	0	0	0	0	0	0
7	0	0	1	0	0	0	0	0	0	0
8	0	0	0	0	0	0	0	0	0	0
9	1	0	0	0	0	0	0	0	0	0
10	0	0	0	0	0	0	0	0	1	0
11	0	0	0	0	0	0	0	0	0	0
12	0	0	0	0	0	0	0	0	0	0
Untreated Check	0	0	0	0	0	0	0	0	0	0

Table 48.--Coccinellidae, adult (A) and larval (L), field population counts per sorghum plant, 58 days after planting, replicate 1.

Treatment	Observation									
	1		2		3		4		5	
	A	L	A	L	A	L	A	L	A	L
1	0	0	0	1	0	2	1	0	0	0
2	0	0	1	0	0	2	2	0	0	0
3	0	0	0	0	1	0	1	0	0	0
4	0	0	0	0	0	0	1	0	0	0
5	1	0	1	0	0	0	1	0	0	0
6	0	0	0	1	2	0	0	0	1	0
7	0	0	0	1	2	0	0	0	1	0
8	1	0	1	0	2	1	0	1	1	0
9	0	0	0	1	0	1	0	0	0	0
10	0	0	1	1	0	0	0	0	0	1
11	1	0	0	0	0	1	0	0	1	1
12	0	1	0	1	1	0	0	1	1	0
Untreated Check	0	0	1	0	1	1	1	4	1	1

Table 49.--Coccinellidae, adult (A) and larval (L), field population counts per sorghum plant, 58 days after planting, replicate 2.

Treatment	Observation									
	1		2		3		4		5	
	A	L	A	L	A	L	A	L	A	L
1	3	0	0	0	0	1	1	1	2	0
2	1	2	1	0	1	0	0	0	1	0
3	1	0	0	0	0	0	2	1	0	0
4	0	0	0	0	0	0	0	0	0	0
5	0	0	0	1	0	0	1	0	0	0
6	0	0	1	0	0	0	1	0	0	1
7	0	0	0	0	0	17	0	1	0	0
8	0	0	1	0	0	0	0	0	0	0
9	2	0	0	0	0	1	0	16	0	0
10	0	1	0	0	1	1	1	1	2	0
11	2	1	1	1	1	0	0	0	2	0
12	0	0	0	0	0	0	2	0	0	0
Untreated Check	1	0	0	0	0	1	0	0	0	0

Table 50.--Coccinellidae, adult (A) and larval (L), field population counts per sorghum plant, 58 days after planting, replicate 3.

Treatment	Observation									
	1		2		3		4		5	
	A	L	A	L	A	L	A	L	A	L
1	0	1	1	0	0	0	0	0	0	1
2	0	1	1	0	0	0	1	0	0	0
3	0	0	3	0	0	0	2	1	0	1
4	1	0	0	0	1	0	0	0	0	0
5	0	0	1	0	1	0	1	0	0	1
6	0	1	0	0	0	0	0	0	1	0
7	0	1	0	1	0	12	1	0	1	0
8	0	0	2	0	0	0	0	0	0	0
9	1	0	1	0	0	0	1	0	1	0
10	0	0	1	0	1	1	1	0	0	0
11	1	0	0	0	0	0	1	1	2	0
12	0	1	0	0	0	0	0	0	0	0
Untreated Check	0	0	2	0	0	1	0	0	1	1

Table 51.--Coccinellidae, adult (A) and larval (L), field population counts per sorghum plant, 58 days after planting, replicate 4.

Treatment	Observation									
	1		2		3		4		5	
	A	L	A	L	A	L	A	L	A	L
1	0	0	1	0	0	0	0	1	0	0
2	0	1	0	6	0	2	0	0	0	0
3	1	0	0	0	0	0	1	0	0	0
4	0	1	0	0	0	0	0	1	0	4
5	1	0	0	0	0	0	0	0	1	0
6	0	0	0	0	1	0	0	0	0	0
7	0	0	1	2	0	0	0	0	2	2
8	0	0	3	13	0	0	0	0	0	0
9	0	0	0	0	0	1	0	0	1	0
10	0	0	1	3	1	0	1	0	0	0
11	0	1	0	0	0	0	0	0	0	1
12	0	0	0	0	0	0	0	0	0	0
Untreated Check	0	0	0	1	0	1	1	0	0	1

Table 52.--Coccinellidae, adult (A) and larval (L), field population counts per sorghum plant, 58 days after planting, replicate 5.

Treatment	Observation									
	1		2		3		4		5	
	A	L	A	L	A	L	A	L	A	L
1	2	0	0	0	1	10	2	0	1	1
2	2	0	1	0	1	0	1	1	0	1
3	0	0	0	0	0	0	0	1	1	0
4	0	0	0	1	0	0	1	0	0	0
5	0	0	0	0	0	0	0	1	0	1
6	0	1	1	1	0	0	1	0	0	0
7	0	0	1	0	0	0	0	6	0	0
8	1	0	0	1	1	0	0	0	0	1
9	0	1	0	0	2	0	0	1	1	0
10	1	0	0	0	2	0	0	0	1	0
11	0	0	2	0	1	0	0	0	1	0
12	1	0	1	0	1	0	0	0	0	0
Untreated Check	1	0	0	0	0	0	0	0	0	0

Table 53.--Parasite mummy field population counts from a single leaf at the third sorghum leaf level, 37 days after planting, replicate 1.

Treatment	Observation 1	2	3	4	5
1	1	3	1	0	1
2	0	0	2	4	3
3	0	1	3	1	0
4	1	0	2	0	2
5	1	0	1	1	0
6	0	0	0	0	0
7	0	0	0	0	0
8	1	0	0	0	0
9	1	0	1	1	16
10	0	3	4	5	7
11	0	0	0	0	0
12	2	8	6	3	2
Untreated Check	2	5	1	2	2

Table 54.--Parasite mummy field population counts from a single leaf at the third sorghum leaf level, 37 days after planting, replicate 2.

Treatment	Observation				
	1	2	3	4	5
1	1	0	3	2	1
2	0	13	0	0	35
3	7	0	6	1	0
4	0	7	5	3	0
5	11	1	0	0	0
6	0	0	0	0	0
7	1	0	1	0	0
8	0	0	0	0	0
9	0	0	0	0	0
10	4	5	0	2	0
11	0	0	0	0	0
12	5	7	3	1	0
Untreated Check	6	1	0	0	3

Table 55.--Parasite mummy field population counts from a single leaf at the third sorghum leaf level, 37 days after planting, replicate 3.

	Observation				
Treatment	1	2	3	4	5
1	1	1	1	0	0
2	5	25	3	1	3
3	0	1	1	1	3
4	1	0	0	0	1
5	0	1	1	0	1
6	0	0	0	0	0
7	1	0	4	1	0
8	0	0	0	1	0
9	2	0	0	0	0
10	0	0	1	2	0
11	0	0	0	0	0
12	0	16	2	1	1
Untreated Check	7	6	3	10	1

Table 56.--Parasite mummy field population counts from a single leaf at the third sorghum leaf level, 37 days after planting, replicate 4.

Treatment	Observation 1	2	3	4	5
1	33	7	2	0	0
2	0	4	1	2	5
3	7	2	15	20	4
4	4	4	17	3	1
5	1	1	0	1	3
6	0	0	0	0	0
7	5	2	0	0	0
8	0	0	0	1	0
9	3	4	0	0	0
10	0	0	2	3	2
11	0	0	0	0	1
12	15	5	3	2	0
Untreated Check	0	0	0	0	10

Table 57.--Parasite mummy field population counts from a single leaf at the third sorghum leaf level, 37 days after planting, replicate 5.

Treatment	Observation				
	1	2	3	4	5
1	5	1	1	0	0
2	3	8	0	7	14
3	4	30	1	3	0
4	2	1	1	1	7
5	0	0	1	0	1
6	0	2	2	0	0
7	0	0	1	0	0
8	0	4	1	10	0
9	0	0	2	0	1
10	7	2	0	1	4
11	0	0	0	0	0
12	0	7	2	4	4
Untreated Check	0	18	5	5	1

Table 58.--Parasite mummy field population counts from a single leaf at the third sorghum leaf level, 58 days after planting, replicate 1.

Treatment	Observation				
	1	2	3	4	5
1	7	10	49	8	83
2	145	75	82	125	0
3	70	126	122	12	3
4	0	0	0	0	0
5	2	24	87	35	63
6	0	0	0	1	1
7	20	3	1	1	20
8	11	2	7	5	4
9	43	60	6	11	1
10	54	5	87	56	59
11	0	0	0	0	0
12	65	145	82	115	157
Untreated Check	16	100	205	127	130

Table 59.--Parasite mummy field population counts from a single leaf at the third sorghum leaf level, 58 days after planting, replicate 2.

Treatment	Observation				
	1	2	3	4	5
1	85	70	140	70	125
2	55	115	120	170	110
3	190	40	50	20	100
4	180	100	70	110	130
5	25	30	110	260	110
6	6	8	6	20	10
7	310	270	140	20	110
8	31	55	50	5	10
9	1	1	10	0	2
10	220	110	190	120	210
11	0	0	3	0	1
12	200	130	90	35	120
Untreated Check	10	3	10	20	5

Table 60.--Parasite mummy field population counts from a single leaf at the third sorghum leaf level, 58 days after planting, replicate 3.

	Observation				
Treatment	1	2	3	4	5
1	30	4	9	14	20
2	35	95	9	6	0
3	9	35	106	76	153
4	0	0	1	0	0
5	18	18	22	20	1
6	0	1	9	2	0
7	17	32	23	28	11
8	27	8	1	3	3
9	0	0	0	7	0
10	39	30	8	1	4
11	0	0	0	0	2
12	10	56	27	36	81
Untreated Check	0	5	74	19	70

Table 61.--Parasite mummy field population counts from a single leaf at the third sorghum leaf level, 58 days after planting, replicate 4.

Treatment	Observation				
	1	2	3	4	5
1	90	140	60	75	90
2	70	30	130	190	100
3	83	78	0	0	25
4	0	0	4	0	0
5	70	30	20	9	35
6	2	0	1	0	0
7	18	15	8	14	55
8	15	140	20	60	60
9	5	1	3	0	0
10	6	18	9	10	90
11	2	0	0	0	12
12	210	190	130	90	50
Untreated Check	60	35	75	55	70

Table 62.--Parasite mummy field population counts from a single leaf at the third sorghum leaf level, 58 days after planting, replicate 5.

Treatment	Observation				
	1	2	3	4	5
1	24	35	33	60	12
2	42	84	37	63	14
3	8	46	12	22	35
4	1	2	8	2	55
5	4	41	27	36	8
6	5	16	13	1	5
7	2	12	3	7	8
8	11	9	14	34	49
9	1	0	1	4	0
10	3	3	2	2	13
11	0	0	0	0	2
12	0	0	0	0	0
Untreated Check	1	0	0	0	0

Table 63.--Least-squares analysis of variance of field insect populations on grain sorghum, Southeast Research Farm, Beresford, South Dakota; 31, 37, and 58 days after planting.

Greenbug, 31 days

Source	df	SS	MS	F
Treatment	12	16247.93	1353.99	1.40
Replicate	4	18555.10	4638.77	7.10**
Treat X Rep	48	46089.94	960.21	1.47*
Error	260	169973.60	653.74	

Greenbug, 37 days

Source	df	SS	MS	F
Treatment	12	32454.44	2704.54	3.18**
Replicate	4	15587.98	3897.00	6.60**
Treat X Rep	48	40774.58	849.47	1.44*
Error	260	153622.40	590.86	

Table 63 (Continued).--Least-squares analysis of variance of field insect populations on grain sorghum, Southeast Research Farm, Beresford, South Dakota; 31, 37, and 58 days after planting.

Adult Coccinellidae, 37 days

Source	df	SS	MS	F
Treatment	12	0.23	0.02	0.56
Replicate	4	0.35	0.09	2.55*
Treat X Rep	48	1.43	0.03	0.86
Error	260	9.00	0.03	

Larval Coccinellidae, 37 days

Source	df	SS	MS	F
Treatment	12	3.03	0.25	1.56
Replicate	4	0.97	0.24	1.50
Treat X Rep	48	6.69	0.14	0.86
Error	260	42.08	0.16	

Parasite mummies, 37 days

Source	df	SS	MS	F
Treatment	12	60.52	5.04	6.3**
Replicate	4	4.85	1.21	1.81
Treat X Rep	48	38.71	0.81	1.21
Error	260	173.97	0.67	

Table 63 (Continued).--Least-squares analysis of variance of field insect populations on grain sorghum, Southeast Research Farm, Beresford, South Dakota; 31, 37, and 58 days after planting.

Greenbug, 58 days

Source	df	SS	MS	F
Treatment	12	31.21	2.60	1.83
Replicate	4	5.61	1.40	1.29
Treat X Rep	48	68.11	1.42	1.30
Error	240	282.94	1.09	

Adult Coccinellidae, 58 days

Source	df	SS	MS	F
Treatment	12	1.29	0.11	1.21
Replicate	4	0.64	0.16	1.81
Treat X Rep	48	5.21	0.11	1.22
Error	260	23.10	0.09	

Table 64.--Least-squares means of insect populations for which the least-squares analysis of variance did not indicate treatment significance.

Treatment	Least-squares means		
	Greenbug, 31 days	Adult Coccinellidae, 37 days	Larval Coccinellidae 37 days
Untreated Check	17.40	0.79	0.74
1	22.80	0.77	1.02
2	24.24	0.79	0.85
3	19.80	0.79	0.71
4	14.40	0.81	0.71
5	14.04	0.79	0.73
6	7.96	0.75	0.71
7	17.56	0.79	0.71
8	10.76	0.77	0.71
9	22.00	0.75	0.71
10	26.48	0.81	0.71
11	1.08	0.73	0.80
12	24.16	0.82	0.92

Table 64 (Continued).--Least-squares means of insect populations for which the least-squares analysis of variance did not indicate treatment significance.

Treatment	Least-squares means		
	Greenbug, 58 days	Adult Coccinellidae, 58 days	Larval Coccinellidae, 58 days
Untreated Check	1.14	0.91	0.93
1	1.60	0.98	0.99
2	1.74	0.98	0.97
3	1.27	0.95	0.79
4	0.91	0.79	0.83
5	1.57	0.89	0.79
6	1.32	0.89	0.82
7	1.49	0.88	1.19
8	1.43	0.95	0.90
9	0.85	0.90	0.97
10	1.72	1.00	0.88
11	0.83	1.01	0.85
12	1.06	0.85	0.79